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MALARIA AND SOCIETY[1]

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PROGRESS

Discussing the effects of that "unseen and still unknown poison," called *malaria*, on the United States of America, John Maccullock wrote in 1829, "What the fate of much of this new country may ultimately be in this respect, it is difficult to foresee . . ." He then ventured the suggestion that "no changes and no cultivation will ever bring into a state of salubrity, a country so abounding with alluvial plains, even in the interior, and so extensively the produce of its numerous and enormous rivers." In those days the fevers attributed to malaria were endemic, and in some areas severely so, over most of the eastern two-thirds of the country and in the central valley of California.

As recently as 1912–1915, when the U. S. Public Health Service surveyed 12 southern states, it was estimated on the basis of the parasite index that a million cases of malaria were occurring annually in a population group of 25 million, with incidence rates as high as 40.9 per cent in the Mississippi Delta. Ten years later, in 1923–24, there were still numerous areas of intense malaria in the South. I recall clearly two cases of blackwater fever which occurred in Lee County, Georgia, during the summer of 1924 when I was with Doctor S. T. Darling at his Leesburg Station for Malaria Studies.

How different the situation today! The tentative count of all reported cases of malarial fevers in the entire country in 1949 was less than 5,000. The Public Health Service is seeing so few cases that it has appealed to our Society to assist in the formulation of criteria for determining the rapidly approaching end point of endemic malaria in the United States.

Elsewhere in the world similar changes are evident. Witness Greece, for example. Ever since the days when Hippocrates wrote about men who drank marsh water and thereby acquired large spleens, malaria dominated the scene. The entire rural life of the country was affected. In 1905 it was estimated that in a total population of two and one-half million persons there were a million cases of the intermittent fevers each year, with some 6,000 deaths. Yet today, after a disastrous period of warfare, which must have greatly intensified the potential, malaria in Greece is a disease of relatively minor importance, thanks to DDT energetically applied. So, too, in Italy, the classic home of paludism since the days of the Caesars, this disease, although it had tremendous incidence during World War II, is now fading into insignificance, thanks again to the systematic application of residual spray. Sardinia and Sicily, notoriously infested with "the bad airs," are now practically non-malarious.

[1] The President's Address, delivered at the 33rd Annual Meeting of the National Malaria Society, Savannah, Georgia, 7 November 1950.

In Venezuela, Brazil, British Guiana, Argentina, Cyprus, Mauritius, Ceylon, and parts of India, to mention some outstanding examples, malaria at last has been checked and in some places already has a phenomenally low incidence. The way to effective control throughout the world is wide open.

In 1936, after some 10 years' residence in the tropics, I expressed the opinion that man did not know how to control malaria in the rural tropics at a price financially feasible on a routine community-budget basis. In those days this cost ranged from 50 cents to $4, or even more, per capita per year, and I estimated that it must not exceed approximately 10 cents per capita to be feasible in the average rural tropical village, at least in the Orient. Today the new methods can be applied so cheaply that few communities, however badly afflicted, are too poor to pay for effective malaria control. A notable example of what is now possible is seen in Bombay State, where currently a malaria control group, under the capable direction of D. K. Viswanathan and T. Ramachandra Rao, is spraying DDT in two million houses, thus protecting nine million people, at a cost of 2.7 million rupees a year. This represents only about 6.3 U. S. cents per capita per year, considerably less than the expense which the disease itself puts upon a community.

PESSIMISM

Surely there has been remarkable progress. The public concerned has seemed immensely pleased to be relieved from annual chills and fever, and from doctors' and undertakers' bills. One might have expected universal satisfaction, even jubilation, at such results. But this has not been the case.

For example, an experienced and capable leader in the field of public health, pointing out that the effective island-wide malaria control program in Ceylon has apparently reduced general mortality by one-third within a few years, recently used the expression, "the hazards of health promotion by modern weapons." Also a short time ago, the editor of a very sound medical journal complained that, "To eliminate disease as one of the natural checks and balances on a nation's population is to be confronted with other checks, such as famine, lack of *Lebensraum*, and war— forces exploited by Hitler and Mussolini." The editor rightly commented, apropos of the Federal Point Four Program, that our vision should be broader than that of Johnny Appleseed. "A beneficent impulse to prevent disease instead of planting apples is not enough in the second half of the twentieth century . . . Good public health is more than the absence of disease. It is a part of good ecology." We certainly endorse this sentiment for it is obvious that human ecology and human welfare are intimately and intricately related. But then the editor makes the statement that, "Liberation from disease without preparation for a balanced ecology within the domain of a people's environment may just as well be the key to over-population, unemployment, communism and war as to peace, prosperity, and perfection." The editorial ended with the statement that "Man has already opened Pandora's box and found that the nucleus of the atom lies within. He still has the lid of the box open when he releases all the mechanisms that limit the increase of human population, without anticipating and preparing for the consequences of his own good intention."

These remarks, and quotations which could be given from other sources, seem to

imply that unless we can prepare *in advance* for such problems as overpopulation we must slow down on public health efforts lest we do harm. This implication, besides being disturbing on humanitarian grounds, seems to me to lack perspective. It appears to reflect emotions aroused by pessimists like William Vogt. The latter's argument, and that of other neo-Malthusians, seems to be that unless we allow natural forces to decimate populations, we may as well give up hope of continuing civilized life. Vogt criticizes the "dangerous doctor" who tries to prevent illness and death. Sanitarians, he writes, ". . . set the stage for disaster; then, like Pilate, they wash their hands of the consequences." Vogt seems to believe that the first step toward the world's salvation should be to stop malaria control and other public health work—not, of course, in the United States, but only in far distant places among the Chinese, the Indians, and the Africans!

Obviously, malaria control and other health improvements in some countries may outrun social developments. They do not have to travel very fast to do so. But this leadership, while it emphasizes the need for giving more attention to other aspects of human welfare, cannot logically be designated a cause of imbalance. We must not be deceived by that vivid word, "balance." There is the sort of equilibrium obtained in a delicatessen store by removing a pickle from the heavier side of a scales, but this is not in any way analogous to obtaining a balanced ecology in human society. *Withholding public health cannot possibly restore a balance which never existed in the past and which never can be had in the future without public health.* Improved agricultural methods, more industrialization, better general education, intelligent control of population growth, these have been obvious social needs for a very long time and must have much greater emphasis. But how can they be added in any volume to a society which lacks average good health? Widespread family planning becomes possible only when standards of community health and living are raised considerably above minimum subsistence levels. Large families are much striven for where economic levels are low, because children are a source of cheap labor; they are the poor man's wealth. People overmultiply largely *because* they are underprivileged.

Along with the implication that we, as sanitarians, are pushing too hard, and that we should ease off until population pressures are lessened, is the disconcerting opinion that we must expand our concepts because Public Health "is really applied Social Science," and that when we as sanitarians are unwilling to deal with overpopulation by relaxing our efforts, or by other methods, we are "tampering" (of all words!) with one side of an equation, and thus are doing harm. For the record, let us follow Winslow and define Public Health as the science and art of preventing disease, prolonging life, and promoting physical and mental health and efficiency through organized community efforts. Then let us adapt Willits' definition of the Social Sciences, and say that they constitute a body of disciplines concerned first with bringing the rigorous methods of scientific analysis to the problems of social behavior, whether in economics, in government, in international relations, or in human relations, and secondly with the application of scientific findings in these matters.

Surely in this age, when greater and greater specialization is absolutely necessary if one is to become expert in his field, it is questionable logic to argue that malariol-

ogists and sanitarians should also be ecologists, economists, and sociologists, except to that normal extent to which the practitioners of all the technologies involved in human ecology must comprehend each other's aims and procedures. Few would deny the need for a closer meeting together of the biologic, economic, medical, and social intellectual disciplines which bear on the problems of human ecology. Public health in general, and malariology in particular, have had the advantage of well-focussed objectives, well-defined specialties, and well-trained personnel. They have led the way! Instead of suggesting that sanitarians should become social scientists, how much better to use public health as that rallying point around which, as suggested by Raymond Fosdick, men of differing cultures, disciplines, and faiths may combine, 'oining their efforts in the common cause.

More and more, leaders in sanitary and social sciences find themselves in agreement that public health work offers an opportunity for a much wider utilization of social scientists than has yet been attempted. It is generally recognized that social science has proceeded to the point, both in development of men and in growth of scientific knowledge, where its ability to contribute can be widely employed. The last war demonstrated that public health is one of the banners under which application of certain forms of social science could grow. Indeed, in many public health projects there might be profitably employed a carefully selected social scientist, of the social engineer type, to aid in meeting the social problems which always arise. This exposure to practical reality should also serve the growth of social science.

The increasingly urgent and difficult problem of population densities requires cooperative study and positive action, and it will not be solved by neglecting to attack disease at home or abroad. Pooled intelligence is required, not passive dependence on those capricious natural forces which indiscriminately sacrifice human lives and cultural institutions. When teamwork becomes the rule, then there will be fewer complaints about dangerous doctors upsetting a balance, tampering with an equation, opening a Pandora's box, and creating hazards of good health!

OPTIMISM

So much for the pessimists! What about the optimists? For optimism as well as pessimism can be excessive. What about our colleagues who say that malaria has ceased to be important and that no more funds need be budgeted for malaria research? Or those who tell us that malariologists, like the dodo birds, are extinct but don't yet know it? Or that what one should do in any malarious country is simply to eradicate the mosquitoes and not temporize with recurring control measures?

No one doubts that it is possible to eliminate all transmission of malaria in an area by reducing the numbers of the vector anophelines until they are below a certain critical density, the magnitude of which depends on the natural history of mosquito and of man. But most vector control measures must be repetitive. Hence, by contrast, a semantic appeal lies in the term *vector eradication*. This suggests the ending of a project by one complete effort and thus sounds much more attractive than mere *vector reduction*. Stimulated by the brilliantly successful expulsion of *Anopheles gambiae* from Brazil and from Egypt, some observers have come to advocate vector eradication as the method of choice to accomplish malaria control almost anywhere.

It is now becoming clear that only under special conditions is *Anopheles* eradication financially sound. In most situations the elimination of a mosquito species is a costly and difficult undertaking which, even if successful, requires thereafter repetitive maintenance measures. On the other hand, the ending of malaria transmission by such a mosquito-reducing measure as residual spraying is simple and relatively inexpensive. True, the measure must be repeated year after year, but it does more than control malaria. It assists in dealing with other household insects so that increasingly it is being demanded by householders, whether malaria is a problem or not. Therefore, most communities, even if they eradicate a malaria vector, will still require residual spraying as well as a quarantine and scouting service and probably some drainage and environmental improvements to guard against reappearance of the malaria vector.

Eradication is a method applicable to special areas. In other words, once again, as so many times before, it has been discovered that no method of malaria control is universally applicable. One must attempt to reach the goal by the most direct way. If, on careful study, eradication seems logical, by all means attempt it. Perhaps the local vector has sharply delimited habitats and is vulnerable to extinction. But if eradication is decided upon, time, money, and authority must be available without stint and without end. Undue optimism may be disastrous.

Another case of questionable optimism concerns DDT. This is evident, for example, in labels on insecticide tins assuring the buyer, in large letters, that the product *"DOES NOT CONTAIN DDT."*

Musca domestica, *Culex pipiens* and *C. tarsalis*, *Aedes communis*, *A. dorsalis*, *A. nigromaculis*, *A. punctor*, *A. sollicitans*, *A. taeniorhynchus*, and *A. vexans* have all developed resistance to DDT in the field. *Musca domestica* has also become resistant to benzene hexachloride, lindane, and chlordane in large control projects. Studies have indicated that it is possible for *A. quadrimaculatus* to develop DDT resistance in a laboratory but it is not at all as notable as that of *Aedes* or *Musca*. Thus far no anopheline species has shown inherent resistance to any insecticide in practical field use. However, most observers believe that theoretically it is quite possible for *Anopheles* in time to display a tolerance to DDT which might be troublesome to malariologists.

At any rate, it is too early for complaisance. Malaria still ranks high in the list of man's afflictions and it remains a crippling reality to vast numbers of people in warmer lands. As we become more and more involved in such areas, through military and "Point Four" activities, we may expect the importance of overseas malaria to become clearer. We shall also have to be watchful against imported malaria and its possible spread in the United States for many years to come. There was very little spread in this country of the malaria contracted overseas in World War II because we were so well prepared to prevent it. But one should not assume, as too many do, that DDT has solved all the problems, and that it is illogical for scientists to pay more attention to this disease, needless for sanitary engineers to have further concern over adjusting the environment to prevent anopheline breeding, and wasteful for administrators to budget funds for malaria studies.

HOPE

What then of the future? We realize that malariology is but a small area in the combined sciences concerned with human ecology and welfare, and we know that the sociologic impact of malaria varies greatly throughout the world. To some peoples, as in parts of India and the Middle East, malaria has been a chronic and overwhelming handicap which has made economic and social progress impossible. In other lands, as in the United States, malaria has been a nagging nuisance, not sufficiently severe to stimulate rapid control, but nevertheless until recently doing steady damage to a considerable percentage of our citizens. In still other regions, as in Canada, the disease has had indirect and almost imperceptible effects.

In some regions, as in central Africa, malaria control has been too limited to have any measurable influence on the lives of the people and there is some debate as to what the nature of such effects would be. In other places, as in Sardinia, the current malaria control project has removed an ancient barrier, thus opening up thousands of acres for agricultural development and stimulating industry, hydroelectric schemes, fisheries, tourism, and many other kinds of endeavor. In still other places, as in Ceylon, malaria control has had an even more dramatic result, so that it has aroused the pessimistic warnings, mentioned above, about "demographic realities."

But, despite such cries of alarm, we still believe that in a modest way we as malariologists are carrying our share of the load up that long and steep road which leads to a world community in which all men and their families will have good health. We are told that, as stated in the World Health Organization constitution, such bounteous well-being is "one of the fundamental rights of every human being, without distinction of race, religion, political belief, economic or social condition." But we think that very likely men must *earn* these benefits and not expect them by inherent right, or by decree, or even by international administrative effort.

So we believe in the practice of helping others to help themselves. We do not believe that either political afflatus or free hand-outs will raise a debilitated, parasite-beaten community to a significantly higher plane of human welfare. Modern public health practice grows in a country by patient training of personnel, by the painstaking development of suitable national and local public health departments, by practical cooperation rather than by charity. Spectacular gifts of new hospitals, or equipment, or tons of DDT, or dozens of assorted experts (for a week or two of high-pressure advice, often in areas previously foreign to them) usually fail to raise standards of health significantly and often engender disrespect and ill will, however generously motivated were the donations.

Steady progress, not haste, is required. If the geologists and paleontologists are correct in their guesses, man has existed for about a million years, and if the astronomers are approximately right in their surmises, man will exist for millions of years longer. Bertrand Russell has called attention to the fact that science as pure knowledge is not over 2500 years old and it has not been a really powerful cause of social change for much over a century and a half, that is, since the Industrial Revolution. Yet we fret because in 150 years science, powerful though it is, has not yet moulded million-year-old mankind into a scientific society! How unreasonable of us to doubt

that in spite of the dreadful upheaval now in its early stages, science will eventually make possible a stable society in which physical, mental, and moral health will be universal and human welfare will be paramount.

So, without pessimism or exaggerated optimism in regard to the social dynamics of malaria control, we point to remarkable progress in the past half century and to the important part taken by our National Malaria Society. We look forward confidently to the day when malaria will be a rare disease, not only at home, but also where it is now most common.

PRELIMINARY EXPERIMENTS IN THE USE OF HOT DDT AND OTHER HALOGENATED HYDROCARBONS FOR RESIDUAL APPLICATIONS

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Disinsectization of international aircraft for the control of medically important insects is accomplished primarily by aerosol applications. Insect control by this means, however, is limited because (1) the aerosols do not have any prolonged insecticidal action, (2) many portions of the aircraft cannot be treated adequately, and (3) both dosage and exposure time are restricted by the type of application.

The use of residual applications would appear to be a valuable adjunct in overcoming the limitations of the aerosols. Residual applications have been made with the insecticide in solutions (Gahan *et al.*, 1944) (Fay *et al.*, 1947), in emulsions (Baker *et al.*, 1947), in suspensions (McCauley *et al.*, 1948), and in dusts (Davis, 1945). Each method of application has introduced certain difficulties in use on aircraft. The solvents, used in solutions and emulsions, had deleterious effects on various aircraft materials or presented fire hazards. The suspensions and dusts presented the problem of unsightly residues. Applications through volatilization present difficulties in uniformity of deposits. Therefore, to overcome the difficulties mentioned, residual applications were tried with DDT and other halogenated hydrocarbons as straight technical materials in a heated liquified state. Since this type of application has shown promise for fields other than aircraft disinsectization, preliminary experiments have been undertaken in its further evaluation.

TECHNIQUE

In liquifying the technical grade DDT for applications, it was first placed in a beaker surrounded by a water bath and stirred until completely melted. The DDT was then sprayed under pressure. In later experiments thermostatically-controlled heating elements, incorporated in the pressure sprayer, allowed for the direct liquification of DDT. One type of sprayer used is shown in figure 1. Several other types are under development.

Spray nozzles delivering fan- or cone-shaped patterns were used in applying the liquid DDT. Initial applications were made with nozzles delivering 0.1 gallon per minute. With this delivery rate it was not possible to obtain deposits as low as 200 mg. DDT per sq. ft. at practical rates of surface coverage. However, initial test panels were prepared by mounting them on a 36-inch-diameter disc with variable speed control. Spray applications to the panels were made through a slot in a shield mounted in front of the disc. With proper disc-speed control, application of 200 mg. of DDT per sq. ft. was obtained with the 0.1 gallon per minute nozzle using 100

[1] From the Technical Development Services, Savannah, Ga., with funds provided in part by the Foreign Quarantine Division.

per cent technical liquified DDT. In later tests, nozzles delivering 0.017 gallon per minute were obtained; and with normal rates of surface coverage, deposits of 200 mg. DDT per sq. ft. were secured. The panel rotator was used in these applications only to insure uniformity of application.

The insecticidal deposits on the test panels were subjected to various conditioning effects. Three-day-old adult *Musca domestica* then were exposed in petri dish-type

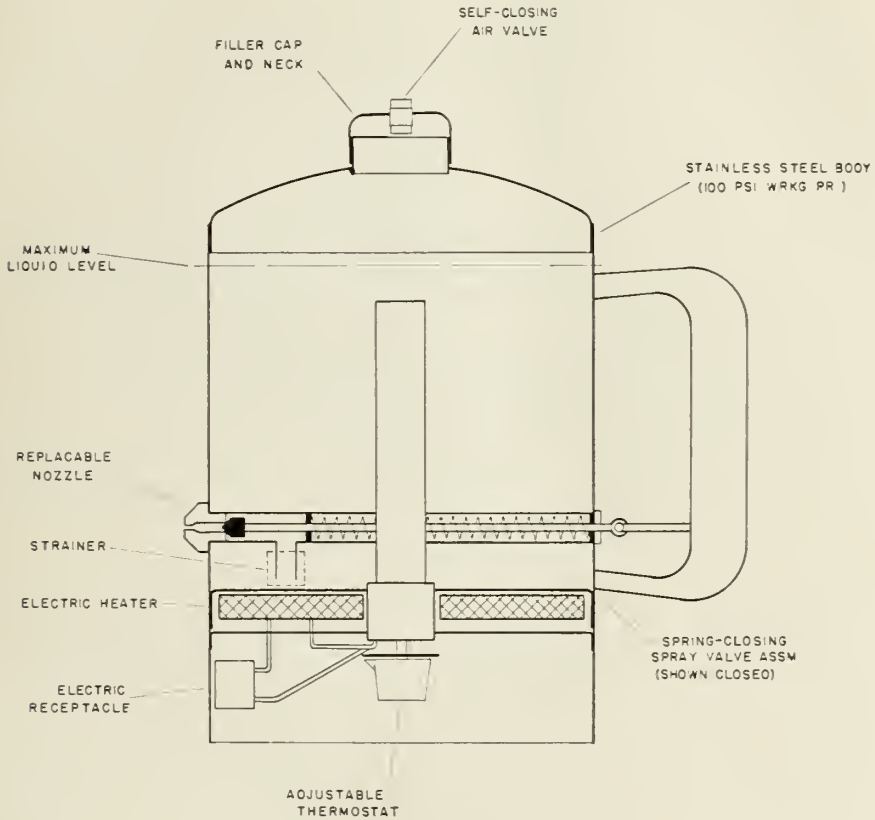

Fig. 1. Thermal Sprayer (Insecticide)

cages (Simmons *et al.*, 1945) or in standard exposure chambers (Fay *et al.*, 1948) to the deposits of various ages. The 24-hour mortalities of the adult females were used to evaluate the residual toxicity.

In special evaluations of vibration effects, a panel vibrator (figure 2) was used. The vibrator consisted of a master panel actuated by a low voltage solenoid energized by 60 cycle A.C. current. The flow of current in the solenoid was interrupted for 5 seconds out of each 10 by breaker points controlled by a cam on a telechron motor shaft. Test panels attached to the master panel were moved up-down and back-forth with an amplitude of $\frac{1}{16}$-inch during each cycle.

RESULTS

Initial tests were made to see if melted DDT could be sprayed under pressure to give toxic residual deposits. One-quarter pound of technical-grade DDT could be

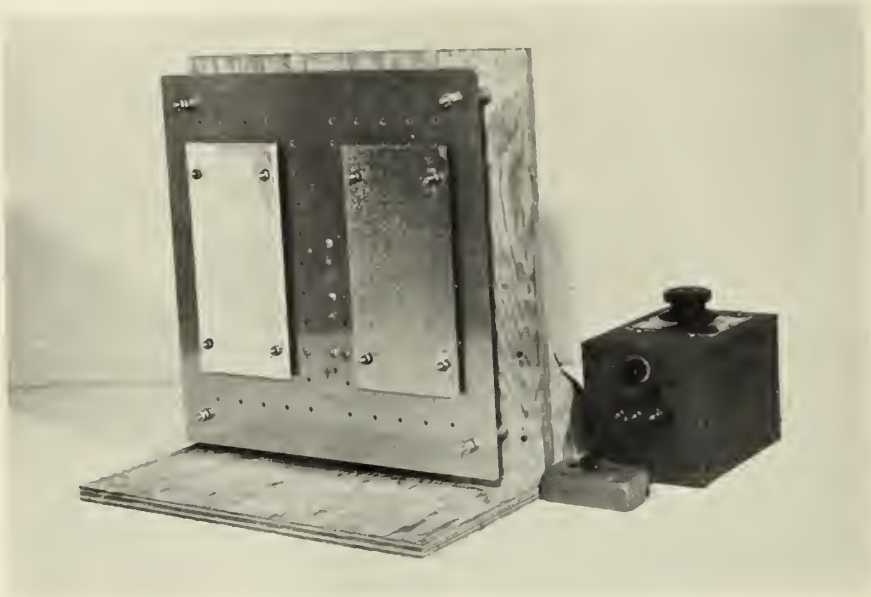

FIG. 2. Test Panel Vibrator (60 cycles per second)

TABLE 1

Biological results from deposits of 200 to 400 mg. per sq. ft. of various melted insecticides sprayed at 95 p.s.i. on glass panels; adult female M. domestica *were exposed for 15 minutes. Each value is the average of six replications*

FORMULATION COMPOSITION	TEMPERATURE (CENTIGRADE)	RESULTS WITH ADULT FEMALE *M. domestica*	
		Immediate Knock-down (per cent)	24-hr. Mortality (per cent)
DDT	97°	96	99
DFDT*	97°	95	100
DDD	97°	64	100
Methoxychlor	97°	97	97
Heptachlor	97°	0	100
Chlordan	97°	10	100
Aldrin	120°	0	99
Lindane	135°	94	100

* p,p′-fluorine analog of DDT.

melted within 15 minutes, with occasional stirring, in a beaker placed in boiling water. When poured into a noninsulated preheated metal sprayer, the melted DDT remained liquid for at least 10 minutes with room temperatures of 70°F or higher.

For proper particle breakup and distribution, spraying pressures of 90 to 100 pounds per square inch were necessary. The melted DDT formed deposits of an adhesive liquid, part of which crystallized within 10 minutes. Other portions of the deposits remained liquid for periods up to 2 months if undisturbed. Adult flies exposed for 15-minute periods to deposits of 200 to 400 mg. DDT per sq. ft. on glass panels showed rapid knockdown and high mortality (table 1).

The tests were extended to include other halogenated hydrocarbon insecticides. It was possible to liquify DFDT, DDD, methoxychlor, and heptachlor using a boiling water bath. Chlordan, though liquid, required the temperature of boiling water to reduce its viscosity. An oil bath was required to liquify aldrin and lindane. It was not possible to melt dieldrin and toxaphene with the above procedure without undue decomposition. The use of small amounts of related materials to reduce the melting point of these materials has been considered. Applications of the melted insecticides were made at the rate of 200 to 400 mg. per sq. ft. and the results (table 1) showed promising residual toxicity in all cases.

All further work in these preliminary studies was confined to DDT formulations. Deposits of 200 mg. DDT per sq. ft. were applied on glass panels using formulations of melted technical DDT, 2.5 per cent DDT suspension made from 75 per cent DDT water-wettable poweder, and 5 per cent DDT-xylene emulsion. During the 12-week period after application, all of these deposits have given complete kill of test *M. domestica* females with 30-minute exposures. A fourth formulation, 5 per cent DDT-xylene emulsion containing 2 per cent rosin, was also tested for comparative purposes. This type of deposit failed to maintain residual toxicity for more than 4 weeks.

Twenty-four hours after application each of the four formulations showed characteristic types of residual deposits before exposure to insects. The melted DDT residual deposits were a mixture of clear sticky viscous droplets and two types of crystals (a) long needle-like crystals arising in groups from a common base, and (b) white hemispherical masses covered with very fine crystals. The wettable DDT deposits were composed of crystal fragments mixed with amorphous carrier particles. The DDT-xylene emulsion deposits were medium length needles many of which projected at an angle from the treated surface. The DDT-rosin deposits consisted of translucent amber hemispheres containing fine crystals and several long-needle-like crystals projecting from the periphery of the hemispheres.

With exposure to the test house flies, the melted DDT deposits showed rapid change: the droplets were smeared over the surface to form scattered masses of fine crystals, the long needle-like crystals were broken off, while the white spherical masses were largely unchanged. The wettable-DDT deposits gradually were removed with successive exposures. In the DDT-xylene emulsion deposits, the crystals projecting at an angle to the treated surface were broken off and lost, but those parallel to the surface were more persistent. In the DDT-rosin deposits, the long needle-like crystals were destroyed leaving only the amber-colored hemispherical crystalline masses.

Thirty-one samples of typical materials used in the interior of passenger aircraft were treated with deposits of 200 mg. of melted DDT per sq. ft. Evaluations of residual toxicity were made 2 weeks later, using the 24-hour mortality of adult *M.*

domestica exposed for 15 minutes. Examinations also were made to determine any visual change in the treated materials before and after exposure to insects. In general, deposits were effective except on the plastic-coated and leather materials (table 2). The deposits caused a slight whitish tinge on all surfaces, which was not noticeable in most cases unless direct comparisons were made. On plastic and leather finishes a few white specks were visible where several droplets of spray had coalesced.

Heavy applications of 400 to 600 mg. melted DDT per sq. ft. were sprayed on Plexiglas and on many types of aircraft insulated electric wire to determine possible detrimental effects. The materials were microscopically examined at various times over a four-week period. When the DDT was removed from the Plexiglas, no signs of pitting or crazing were observed. No deterioration was detected in any of the insulated wires treated.

Insects have been detected in such places as the baggage compartments, cupboards, under floor boards, and in the tail assembly on intercontinental aircraft. The surfaces of these areas are largely metal and are subjected to considerable vibration and rubbing action so that the adhesive qualities of residual deposits become important.

Sheet aluminum panels, 4 by 8 inches, were sprayed to give deposits of 200 mg. DDT per sq. ft. using melted DDT and a water-wettable suspension. After a 1-week drying period, test panels from each preparation were mounted on a panel vibrator and shaken for 50 per cent of the time over periods of 18, 60, and 100 hours. Visually, it was evident that some of the water-wettable DDT was shaken from the test panels, but this amount was not sufficient to influence the residual toxicity of the panels.

Residual toxicity was measured by exposing vibrated and nonvibrated panels to adult *M. domestica* for periods of 5, 10, and 15 minutes. Complete 24-hour kills were obtained in all tests.

Under rubbing action the melted DDT deposits were considerably more adhesive to the sheet aluminum than were the water-wettable deposits. The result of one stroke with a cloth using medium hand pressure was determined. The melted DDT adhered to the surface like semidry paint, while the water-wettable deposits were almost entirely removed.

If the use of melted DDT were extended to field control programs, the weathering ability of the residual deposits would be an important factor. Preliminary determinations have been made, therefore, to measure the residual effectiveness of various DDT deposits exposed to outside weathering. Deposits of 200 mg. DDT per sq. ft. were applied on glass panels using (1) melted technical-grade DDT, (2) a 5 per cent DDT-xylene emulsion, (3) a 2.5 per cent DDT suspension made with 75 per cent DDT water-wettable powder, and (4) a 5 per cent DDT-xylene emulsion containing 2 per cent rosin. After a 48-hour drying period, the test panels were exposed to outside weathering for 1, 4, 8, and 12 weeks. The results (table 3) showed the DDT-rosin deposits to be effective for at least 12 weeks and the melted DDT for 8 weeks; while the DDT emulsion and water-wettable DDT showed less long-lasting effectiveness.

The difference in the residual effectiveness of DDT-rosin deposits held inside (previously noted) and those exposed to weathering (table 3) was very marked. Microscopic examination of the deposits revealed that a considerable portion of the

TABLE 2

Effects of 200-milligram deposits of melted DDT per square foot on interior materials (aircraft), and the 24-hour mortalities obtained when M. domestica *were exposed to these deposits for 15 minutes*

MATERIALS				AV. PER CENT KILL *M. domestica*	
Type	Use	Color	Visible Change	Male	Female
Wool Gabardine	Curtain	Blue-purple	Very minor*	100	100
		Medium brown		100	100
		Nile green		100	100
		Powder blue		100	100
		Rose red		100	100
Wool pile	Upholstery	Blue-gray		100	100
Wool	Chair Upholstery	Light brown		100	100
	Carpet	Medium gray		100	100
	Blanket	Medium green		100	98
		Blue		100	100
Cotton fabric	Safety belt	Gray-green		100	100
Flightex	Headrest cover	White	None	100	100
Fiberglas	Insulation	Peach		100	100
Wool glass		Dirty white		100	98
Cloth liner	Glare curtain	Olive drab	Very minor	100	94
Nylon	Bag cover	Medium green		100	91
	Life-raft cover	Canary yellow		100	89
Plastic-covered fabric	Lavatory lining	Light gray	Few pronounced white deposits	91	10
	Cabin lining			79	20
Leather	Seat arm rest	Green		72	3
	Stewardess seat	Slate gray		69	2
Plastic-covered Fabric	Lounge lining	Robin-egg blue		69	17
	Cabin lining	Slate		38	0
	Cockpit lining	Black		7	0
	Pilot seat cover	Mulberry		6	0
	Cabin lining	Light brown		3	0
		Gray-blue		0	0
		Blue-purple		0	0
	Cockpit lining	Brown		0	0
	Chair facings	Light gray	Very minor	3	0
Fiberglas Cloth	Water tank cover	White	None	0	0

* Treatment not perceptible unless comparison was made directly with untreated materials.

DDT was held in the rosin droplets. Under inside conditions this DDT was shielded from the insects by the rosin. With outside weathering the rosin was gradually dissipated and left behind relatively fresh deposits of DDT available to the insects, hence showing prolonged residual action.

TABLE 3

Mean 24-hour mortalities (per cent) of adult female M. domestica *exposed for 30 minutes to various DDT deposits at different intervals after application. Each value is the average of four replications*

DEPOSITS	AGE OF DEPOSITS (WEEKS)			
	1	4	8	12
Melted DDT	100	100	75	3
DDT emulsion	100	99	6	—
Wettable DDT	100	51	4	—
DDT and rosin	100	100	100	100

DISCUSSION

These preliminary studies on residual applications of melted DDT show sufficient promise to warrant its trial in passenger aircraft as an adjunct to aerosol applications. Since melted DDT is relatively ineffective as well as somewhat unsightly on plastic-coated materials and leather, treatment of these surfaces should be avoided. Residual applications should be confined to baggage compartments, fabric and certain metal surfaces in the passenger compartment, the wheel wells, and the tail assembly. It is believed that residual applications will have the following advantages: (1) insects knocked down by aerosols would fall on treated surfaces (carpets and seats), (2) insects lighting on residually treated surfaces would be stimulated to fly (Fay and Sheppard, 1949) and thereby increase the effectiveness of subsequent aerosol treatments (David *et al.*, 1946) and (3) insects in areas of the aircraft not receiving aerosol treatment would be exposed to toxic residual deposits.

The exploratory experiments with melted DDT and other halogenated hydrocarbon insecticides indicate the application of melted insecticides may be extended to fields other than aircraft disinsectization. By utilizing this melting and spraying technique, the usual formula ingredients such as emulsifiers, adhesive agents, and solvents are eliminated. This elimination offers considerable savings in costs of formula ingredients, mixing equipment, and transportation. Since the insecticides are applied in an undiluted state, the size of the equipment may be substantially reduced. For example, a 1-quart sprayer equipped with a 0.017 gallon per minute nozzle and containing melted DDT would replace a 5-gallon sprayer with a 0.2 gallon per minute nozzle used to apply 5 per cent DDT formulations.

Certain of the insecticides, aldrin, dieldrin, lindane, and toxaphene, could not be melted over boiling water satisfactorily for spraying. For these and some additional insecticides, the use of various semi-eutectic mixtures is being investigated and will be reported later.

In view of the adhesive and weathering qualities of melted DDT deposits, and

the elimination of possible solvent or carrier damage to vegetation, this type of application may find extensive use in agriculture. Furthermore, some preliminary studies indicate that melted DDT may be used as an effective space spray.

SUMMARY

A method has been developed for melting DDT and spraying the liquified product to obtain residual deposits. The method has been shown to be applicable to DDD, DFDT, methoxychlor, chlordan, heptachlor, aldrin, and lindane.

In comparisons with 5 per cent DDT-xylene emulsion and 2.5 per cent water-wettable DDT, the deposits of 200 mg. melted DDT per sq. ft. showed excellent residual effectiveness over a 12-week period in the laboratory.

Deposits of 200 mg. melted DDT per sq. ft. on glass panels were exposed to outside weathering and produced 24-hour mortalities of 75 per cent or better (adult *Musca domestica*) for a period of 8 weeks. This was inferior to deposits from a DDT-rosin formulation but superior to deposits from DDT-xylene emulsion and from water-wettable DDT.

Applications of melted DDT were tested on 31 typical interior-finishing materials for passenger aircraft. A minimum of discoloration was encountered on all materials except certain dark-colored plastic-coated and leather materials. Twenty-four-hour mortalities of adult *M. domestica* after 15-minute exposures were 85 per cent or higher for all materials except the plastic-coated materials. Applications of 400 to 600 mg. melted DDT per sq. ft. sprayed on Plexiglas and insulated electric wires did not cause any visible damage.

Deposits of melted DDT on sheet aluminum showed excellent adhesion when subjected to severe vibration and rubbing. In contrast, visible amounts of water-wettable DDT were removed from sheet aluminum under vibration and the deposits were almost entirely eliminated by a single rubbing stroke.

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RESUMEN

Se ha descubierto un método para derretir DDT y rociar el producto líquido para obtener depósitos residuales. El método se ha demostrado aplicable a DDD, DFDT, "metoxychlor", "chlordan", "heptachlor", "aldrin" y "lindane".

Comparados con una emulsión de DDT y xileno al 5 porciento y con DDT humedecible con agua al 2.5 porciento, los depósitos de 200 miligramos por pie cuadrado de DDT derretido demostraron efectividad residual excelente durante un período de 12 semanas en el laboratorio.

Depósitos en paneles de cristal de 200 miligramos por pie cuadrado de DDT derretido se expusieron a la intemperie y produjeron en 24 horas mortalidades de 75 porciento o mayor (*Musca domestica* adulta) por un período de ocho semanas. Ésto fué inferior a los depósitos derivados de la formulación DDT-resina pero superior a los depósitos de la emulsión DDT-xileno y de DDT humedicible.

Aplicaciones de DDT derretido fueron probadas en 31 materiales típicos de decoración interior para aviones de pasajeros. Se demostró un descoloramiento mínimo en todos los materiales con excepción de ciertos materiales obscuros de cubierta plástica o de cuero. Se produjo una mortalidad de 85 porciento o mayor en *M. domestica* adulta en todos los materiales excepto en los de cubierta plástica después de una exposición de 15 minutos. Aplicaciones de 400–600 miligramos por pie cuadrado de DDT derretido rociadas en "Plexiglas" y en alambres eléctricos aislados no causó ningún daño visible.

Depósitos en planchas de aluminio de DDT derretido demostraron adhesión excelente cuando se les indujo a vibración y fricción severas. En contraste, cantidades visibles de DDT humedecible fueron removidas de las planchas de aluminio bajo vibración y los depósitos fueron casi enteramente eliminados por un roce ligero.

GROWTH CHANGES OF ANOPHELINE EGGS IN WATER AND IN SALINE SOLUTIONS

W. G. DOWNS[1]

(Received for publication 20 November 1950)

Eggs of *Anopheles* have been studied extensively morphologically, and in certain groups they are of distinct value in taxonomy. Aside from this, they have received relatively little attention. They are considered in general as resistant to insecticides, and control of anophelines through primarily ovicidal methods has not been considered as offering good possibilities.

Herms and Freeborn (1920) describe the change in color of anopheline eggs, from pearly white at time of oviposition, progressing through yellow to deep brown or black at the end of 45 minutes, and Christophers (1933) makes similar mention of this phenomenon. No mention is made of change in size.

Observations herein described were carried out, using eggs of anophelines (*Anopheles aztecus* Hoffmann and *Anopheles albimanus* Wied.) reared in the insectary maintained at the Institute of Tropical Diseases in Mexico City, or eggs deposited by wild-caught females (*Anopheles pseudopunctipennis* Theo.), to determine growth characteristics and effects of toxic substances upon the eggs.

Individual gravid mosquitoes were placed in oviposition vials and observed at frequent intervals throughout the day. As soon as an egg batch was noted, the eggs were removed and transferred to a filter paper moistened with the solutions being tested. They were oriented with dissecting needles so that superior and inferior poles of the egg were in the same horizontal plane, and measured, using an ocular micrometer and a magnification in which each 0.1 mm. graduation corresponded to 14.4 microns. Measurements were estimated to the nearest half-graduation. The eggs were remeasured at intervals. Usually, 10 to 15 eggs were selected at random from a batch, to be measured and followed. Between measurements, the eggs were stored in the same position on the filter paper in small corked glass vials, along with a cotton pledget wetted with the same concentration of solution with which the batch was originally started, in an incubator at 22° C. In the recorded observations only fertile eggs are included.

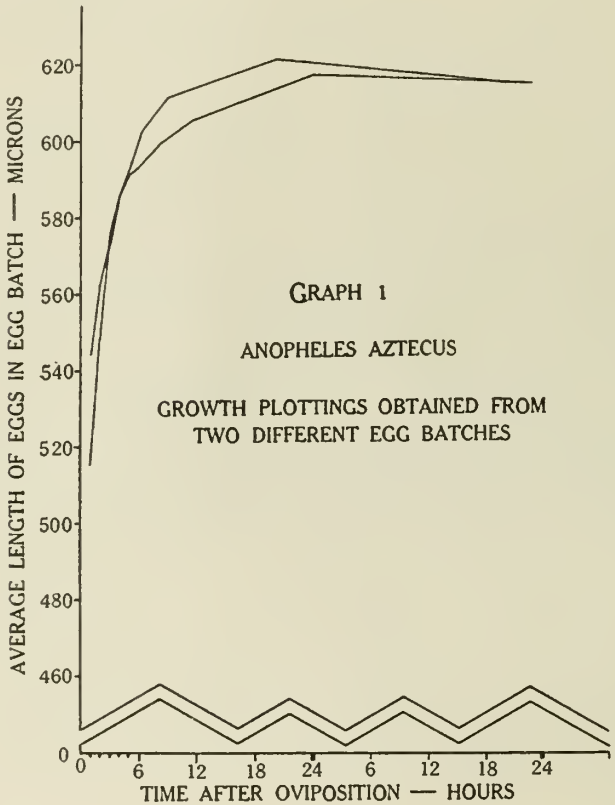
OBSERVATIONS

Graphs 1, 2 and 3 illustrate the growth changes taking place in eggs of *A. aztecus*, *A. albimanus* and *A. pseudopunctipennis* when these eggs are placed upon distilled water. Growth is rapid during the first 24 hours, slows down in the second 24 hours and presumably ceases some 12 to 24 hours before eclosion. Although the graphs illustrate only change in length, measurements taken indicate also increase in width

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in co-operation with the Mexican Health Department.

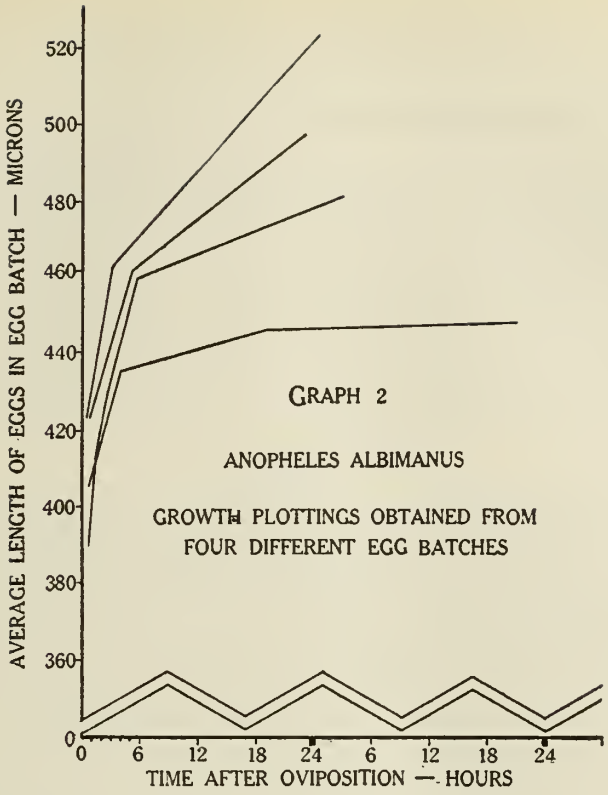
and depth, and it is obvious that the changes represent an increase in volume of egg contents.

Pooled data from many observations, not recorded here, when the data are expressed in terms of ratio of initial length to observed length at later intervals, indicate that the eggs of all three species increase in size at approximately the same rate, and the curve is that of an exponential function. The initial length of eggs increased from 12 to 25 per cent in the period of 24 hours postoviposition, and slightly more in the succeeding 24 hours.



Observations carried out with eggs on papers moistened with concentrations of NaCl ranging from 0.05 N to 1.0 N (sea water may be considered as approximately 0.5 N) indicate that growth of eggs takes place in concentrations up to 0.15 N to 0.2 N, but above 0.2 N no growth occurs, and in higher concentrations the eggs actually shrink in size. Increase in size is inversely proportional to concentration up to 0.2 N to 0.3 N, and decrease in size in higher ranges is directly proportional to increasing concentration.

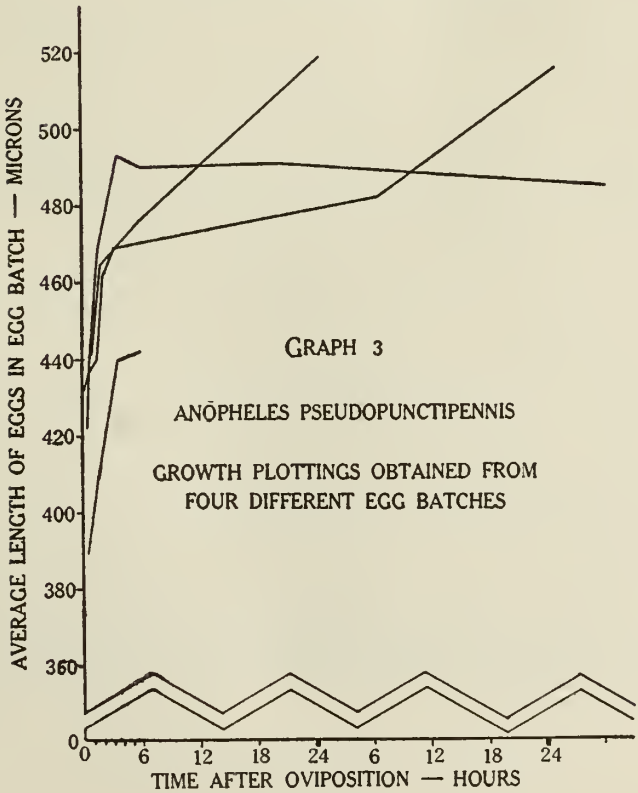
In *A. aztecus*, eggs on concentrations of 0.2 N did not develop embryos. Eggs of *A. albimanus* developed embryos on 0.2 N and 0.3 N NaCl but not in stronger concentrations; and eggs of *A. pseudopunctipennis* developed embryos on 0.18 N



GRAPH 2

ANOPHELES ALBIMANUS

GROWTH PLOTTINGS OBTAINED FROM FOUR DIFFERENT EGG BATCHES



GRAPH 3

ANOPHELES PSEUDOPUNCTIPENNIS

GROWTH PLOTTINGS OBTAINED FROM FOUR DIFFERENT EGG BATCHES

NaCl, only partial development of embryos on 0.2 N, and no development on 0.3 N.

It was also noted that, whereas fertile eggs did not increase in size in concentrations of NaCl greater than 0.15 N to 0.2 N, infertile eggs increased in size in all concentrations up to 1.0 N.

In all of the tests with solutions it is important that the eggs be very fresh (less than ½ hour old). Eggs 2 or 3 hours old are much more resistant to high concentrations of salt.

When very recently laid eggs were placed in contact with DDT and with gammexane (benzene hexachloride) emulsions, they continued to develop and hatch.

DISCUSSION

Johnson (1937) studied the volume changes occurring in eggs of *Notostira erratica* L. (Hemiptera: Capsidae) and noted that for two days after oviposition there was only a slight increase in weight, and from the third day on, a continuous increase in weight. Wigglesworth (1947) illustrates a graph from this study, and considers that the increase is largely due to water absorbed from the external environment. Roonwall (1936), Blunck (1914), Kerenski (1930) quoted by Buxton (1932), in studies on eggs of various insects, describe increase in weight, considering that the increase is due to water absorption. Blunck mentions that the egg membrane can pass water but not dissolved substances, and Kerenski, observing a gain in net weight in eggs of *Anisoplia austriaca* (Coleoptera: Scarabaeidae) in 4 per cent NaCl, states that the water absorption is not simply due to osmosis, but that there is an active absorption of water from surrounding fluids. Beaumont (1948, 1948a), in detailed studies of the eggs of *Rhodnius prolixus* Stal., noted that the unspecialized portion of the shell (and cap) are impermeable to almost all hydrophilic and lipophilic substances, and that penetration of fluids and of toxic substances occurs almost entirely by way of the micropylar structures of the egg. He demonstrates that subchorial membranes become even more impermeable as egg development progresses.

Anopheline eggs begin growth immediately after being deposited, and the growth rate is most rapid early, slowing down by the end of 24 hours. The eggs take up water freely, but apparently the membranes are impermeable to electrolytes. Water is taken up considerably past the range of physiologic saline (0.145 N), but since it is taken up by infertile eggs in concentrations up to 1.0 N and by fertile eggs up to only 0.15 N to 0.2 N, this uptake is not necessarily related to a vital process of the embryo. Indeed, additional water is apparently not essential, since embryonic development and hatching have been observed in eggs on 0.2 N NaCl, which did not increase in size at all.

The sensitivity of freshly laid eggs to relatively low saline concentrations, with failure of the embryo to develop, may explain the failure of many anopheline species to succeed in brackish water. The possibility of an adaptive mechanism in those species which can establish themselves in brackish water merits further investigation.

The destruction of freshly laid eggs by saline concentrations above 0.3 N, is a

mechanism which may help to explain the success of control projects where sea water is let in to fresh-water marshes.

The possibility of attacking anopheline eggs through ovicides does not appear promising, due to impermeability of the membranes.

In using anopheline egg measurements in taxonomic descriptions of species, care should be taken that the eggs being measured are fertile, have been on relatively pure water, and are more than 24 hours old. Otherwise the measurements may be inaccurate as much as 25 per cent. This does not take into account our findings that the mean length of eggs from different egg batches of the same species may also vary widely.

SUMMARY AND CONCLUSIONS

1. Eggs of *Anopheles aztecus*, *A. albimanus* and *A. pseudopunctipennis* increased in length by 12 to 25 per cent in the first 24 hours post-oviposition. There is a concomitant increase in other dimensions of the eggs.

2. Fertile, freshly laid eggs placed upon saline solutions of different concentrations increased in length (inversely to concentration) up to 0.15 to 0.2 N NaCl. In higher concentrations, the eggs shrink (in direct relationship to increase in concentration).

3. Using fertile, freshly laid eggs, embryonic development did not take place in saline concentrations higher than 0.2 N to 0.3 N.

4. Infertile eggs increase in size (inversely to concentration) in saline concentrations as high as 1.0 N.

5. Fertile, freshly laid eggs placed in contact with DDT and with gammexane continued normal development.

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RESUMEN Y CONCLUSIONES

1. Huevos de *A. aztecus*, *A. albimanus* y *A. pseudopunctipennis* aumentaron de 12 a 25 porciento de largo durante las primeras 24 horas después de la puesta. Hubo un aumento simultáneo en las otras dimensiones de las huevos.

2. Los huevecillos fértiles y recién puestos habiendo sido expuestos a soluciones salinas de diferentes concentraciones de 0.15 a 0.2 N de NaCl aumentaron en longitud inversamente a la concentración. En más altas concentraciones los huevecillos se encogen (en relación directa al aumento de la concentración).

3. El desarrollo embrionario de los huevos fértiles, y recién puestos no ocurrió en concentraciones salinas de más de 0.2 N a 0.3 N.

4. En concentraciones salinas tan altas como 1.0 N los huevos estériles aumentan su tamaño inversamente a la concentración.

5. Después de ser expuestos al DDT y al "gammexane", los huevos fértiles y recién puestos continuaron su desarrollo normal.

CHARACTERISTICS OF LARVICIDAL SPRAYS APPLIED BY AIRCRAFT FOR THE CONTROL OF *ANOPHELES QUADRIMACULATUS* ON IMPOUNDED WATER

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(Received for publication 27 November 1950)

During recent years, the mounting cost of labor coupled with the lowering incidence of malaria has dictated modifications in the malaria control program carried out on the reservoirs of the Tennessee Valley Authority. The principal strategy, however, still involves the use of water level management supplemented by shoreline and drainage maintenance for the control of the malaria vector. The marginal band of vegetation has been pushed landward by the accumulative effects of favorable water schedules, and growth control operations have been modified to meet specific conditions encountered in individual reservoirs. In most of the mountain storage pools and in a few main river reservoirs, fall shoreline conditioning has been practically eliminated, while in others maintenance limits have generally been lowered to conform with ecological changes along the margins. Many anopheline producing areas have been eliminated by the construction of permanent works such as topography alteration and diking and dewatering. Other mosquito breeding areas will similarly be "built out" as time progresses. With improved shoreline conditions it has been possible to reduce significantly the labor forces necessary for shoreline maintenance operations and to eliminate boat "mosquito fleets." Greater reliance for satisfactory control of *Anopheles quadrimaculatus* is being placed upon water level management supplemented by airplane application of larvicides.

It appears that there will be a continuing need for an efficient, highly mobile and speedy larvicidal measure to meet emergency conditions as they may arise. On multipurpose reservoir schemes, interruptions in the water level schedules must be anticipated. Storage reservoirs may be filled during the breeding period, and flash floods on tributary streams may enter diked and dewatered areas through spillways provided for the purpose. These conditions, though often of short duration, often result in sharp increases in mosquito production which must be brought under satisfactory control. The Authority maintains four aircraft larvicidal units which stand by in strategic locations in the Valley for immediate use when such conditions arise. These aircraft units are depended upon most frequently when reservoir water levels are in the constant pool phase or when uncleared portions of the basin are inundated. Under uncleared conditions, it is very difficult to obtain adequate mosquito control by the use of larvicides. This report is a continuation of a study of aircraft spray performance designed to lead to improvements in technique in order that the equipment will meet its exacting and difficult task.

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PENETRATION OF DENSE TIMBER CANOPY BY AIRCRAFT SPRAY

In a previous report (Krusé *et al.*, 1949) an attempt was made to analyze the relationship of the number and size of spray particles produced to larvicidal efficiency. For this analysis, the mass of information previously compiled on thermal aerosols was translated in terms of sprays composed of larger diameter particles. These data were summarized in Table 3 (*loc. cit.*). It was shown that with the sprays investigated, patterns were obtained which, in theory, should provide satisfactory mosquito control with greater ease than that obtained with thermal aerosols. In open range studies at flight heights of 25 feet, the performance of the sprays was essentially as had been predicted. However, when DDT in a polymethylated naphthaline (Sovacide 544-B) was applied as a spray at rates of 0.05 and 0.1 lb. per acre, subsequent tests showed that the recovery and bioassay, from beneath a dense timber canopy were grossly inadequate. A prompt restudy of this phase of the problem was thus required.

It may be well to point out at this time that the density and general growth characteristics of the test range employed for this study were much more severe than those of problem areas generally encountered on the various reservoirs. Some problem areas which require routine treatment have taller stands of timber, but observations of their relative densities show the trees to be more widely spaced and that the canopies are usually more lofty and less dense. The test area was composed of second growth timber, mainly hardwoods about 5 to 15 years of age. The spacing of these trees was close, with an estimated density of 2000 to 3000 individuals per acre. Very little natural thinning has taken place, except in the extreme lower story where vines and herbaceous growth were almost completely shaded out. The canopy was dense and complete from the top down to within 10 to 15 feet of the ground. The total height of timber approximated 38 feet. The nature of the canopy and of the terrain required a flight height of about 50 feet in order to maintain a level horizontal course across the test range. Eleven permanent sampling stations were located at 20-foot intervals under the canopy in a line at right angles to the path of flight. The vegetation was undisturbed except for a narrow path which was cleared of dead limbs and vines to facilitate access for bioassay and insecticide recovery studies.

Some 13 test runs were made over the penetration range, using 50 or 100 foot swaths, sprays of varying particle size composition, and differing rates of application, in an effort to arrive at a better understanding of the mechanism of vegetation penetration by spray particles. Satisfactory larval kills across the range were not obtained until the concentrated insecticide was dispersed at rates approaching 0.5 lb. of DDT per acre.

COMPARISON OF THEORY WITH FIELD DATA

According to the theory of dispersion and deposition of aerosols advanced by Johnstone *et al.* (1949), the ability of aircraft-dispersed droplets to penetrate vegetative cover depends upon a number of variables.

The density, depth, and shape of foliage are of great importance. A parameter of these variables has been developed as follows: The efficiency of particle deposition,

ϵ, is a function of the particle mass, the mean wind velocity, the viscosity of air, the particle diameter, and the dimensions and shape of the object upon which impaction occurs. It is assumed that foliage shapes consist of flat plates (leaves) and circular cylinders (branches and twigs), and that the value assigned to the shape for the purposes of calculating ϵ will lie between the values for a flat plate and a circular cylinder. δ and γ are certain density values for the horizontal and vertical directions of the foliage, respectively. The ratio δ/γ is assigned a value of 2, since it is reasonable to assume that the density in the vertical direction is twice that in the horizontal direction. In essence, Johnstone's values of δ and γ may be determined in the field as the distances in feet between the eye and a 1 sq. ft. mirror held in the foliage so as to obtain complete obstruction of the mirror's reflection. In a horizontal or δ direction, Johnstone suggests that 5 feet would be a satisfactory value for dense vegetation and 100 feet for very light vegetation. The depth of cover is controlled by the value of "n" which equals height/δ.

The velocity of subsidence (V_s) is controlled by the diameter and the density of the droplet. It is interesting to note that in the absence of downwash and lateral drift, the ability to penetrate forest cover is independent of both the diameter and density of the droplet, but is proportional to the density of the foliage. Of greater interest is the fact that larger particles will penetrate foliage more efficiently than smaller drops in the presence of lateral drift. This is explained by the fact that faster falling drops have less chance of impaction in a horizontal wind, since they are not carried past as many surfaces as are the smaller droplets. This effect is compensated for by the action of the vertical downwash (V_o) created in the wake of the aircraft. This downwash component will greatly increase the relative penetration efficiency of smaller droplets.

Johnstone (*ibid.* 1949, Fig. 4) has plotted the fraction (f) of the spray which will penetrate to a depth "n" under the influence of a five mph downwash (V_o) in the presence of a 1 mph horizontal drift (u). In addition, the relationships occurring in the absence of horizontal drift and downwash are also plotted. These boundary conditions represent fairly well those occurring in the top of the forest when the Vultee BT-13 spray plane passed over the canopy at 120 mph. Both vertical and horizontal currents were present even though the tests were conducted during times of no measurable wind drift. When the zone of downwash has been penetrated, the particles will next settle in accordance with the law as developed for the absence of both horizontal drift and downwash.

By enlarging Johnstone's Fig. 4 and making due allowances for differences in densities of both forest cover and of the larvicide employed, a comparison could be made to check the field experience against the theory of penetration.

All droplet data have been presented in terms of the new centering constant D_o*, utilizing the graphical solution described previously (Krusé et al., 1949). It will be recalled that when the spectra spread value q is large, the droplets are relatively homogeneous in size; when the droplet range is wide, the value of q is small. In most

* $D_o = \frac{\int d^3 \Delta n}{\int d^2 \Delta n}$ where Δn denotes number of drops, the diameter of which is d.

of the spray patterns employed in this series of tests the q value was never greater than 0.5 while for thermal aerosols q values of 1 or 2 were quite common.

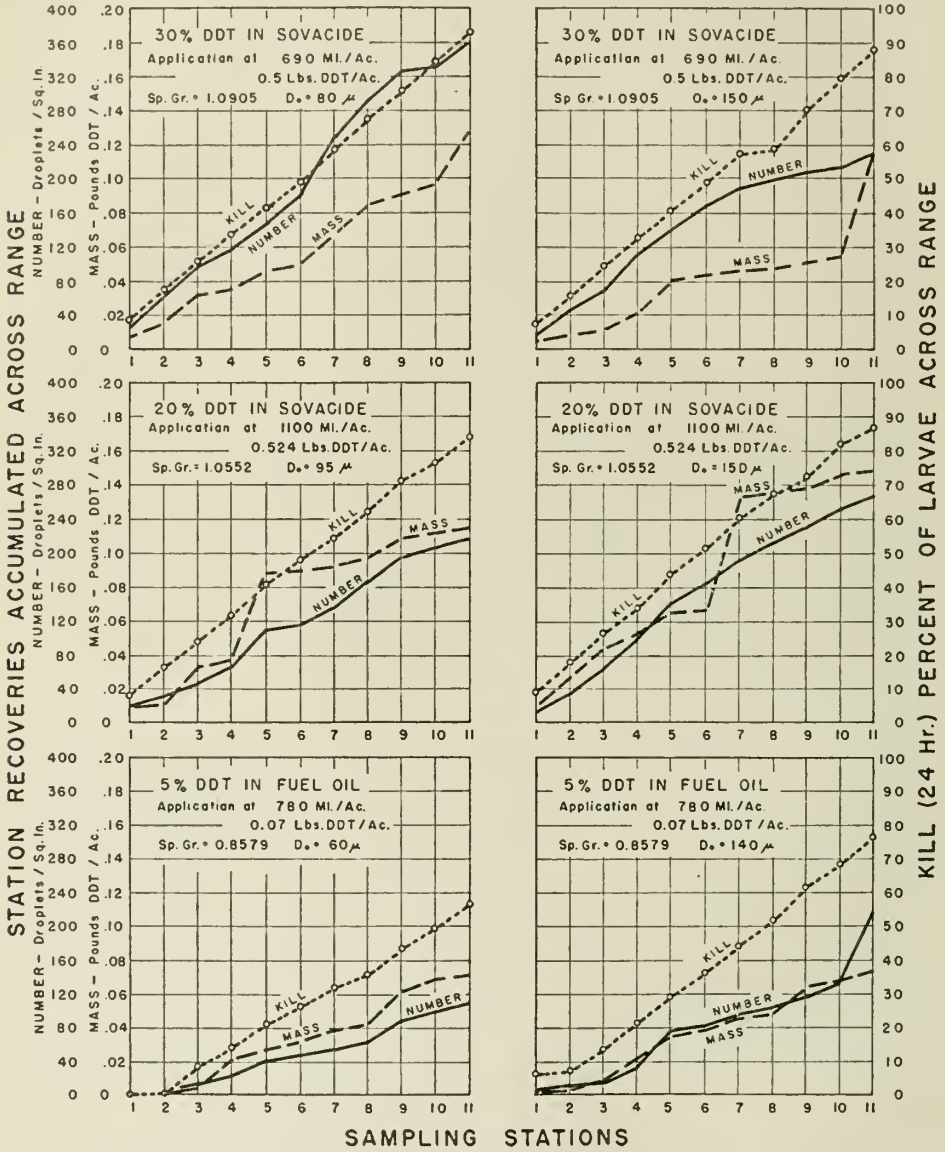

FIG. 1. The particle numbers, mass recoveries, and bioassays of three insecticide formulations applied in two differing spray spectra following the penetration of a high, dense vegetative screen. Applications made from the Vultee BT-13 spray plane flying about 12 feet above the forest canopy and about 50 feet above the test range.

The bioassay, particle number, and mass recovery data from six interesting experiments are presented as cumulative totals per station in Fig. 1. The data yielding

the straightest lines show the greatest uniformity across the swath, while the steepness of the slopes delineates the magnitude of recovery. When dealing with heterogeneous spray spectra, the mass will always show a wider variation than will the number of droplets. All six tests were carried out under the same conditions with approximately the same volume of larvicide being applied for each run. By studying this figure it may be seen that the larvicide of greatest density and having a D_o equal to 80 microns resulted in the best larval kill and greatest recovery of droplets. The 5 per cent DDT-fuel oil larvicide, being the least dense and having the smallest particle size, gave the least number of droplets recovered. In this instance, since the application rate was only 0.07 lb. DDT per acre, as compared with 0.5 lb. DDT or more in the other tests, the larval kills cannot be compared. While there is no significant difference in the number of droplets recovered from the larger particle sizes of both 20 per cent and 30 per cent DDT in Sovacide, the lack of uniformity in amount of recovery is quite apparent. It may be generally concluded that the practical size range of $D_o = 80$ microns is optimum for penetration of dense vegetation, and that the heavier the larvicide the greater will be the uniformity of recovery. The volume discharged must be increased in accordance with the "n" values, to insure that the fraction of droplets actually reaching the water surface will provide a satisfactory and adequate larvicidal dose.

Forest canopies vary considerably. Generally, younger forest stands are the most dense, but are limited in height. A mature forest is taller and usually less dense, but the canopy is arranged in overlapping layers, with very little obstruction from the main trunk level down. It has been noted that the heavier the canopy the less dense will be the understory. The vegetation density values above each sampling station were different. In order to apply the theory of aerosol penetration to our data, three categories of density were established, utilizing the best judgment of existing field conditions.

Conditions for recovery of spray droplets were characterized as follows:

$$\text{Minimum } \delta = 5 \text{ feet for the top 15 feet; } n = \frac{15}{5} = 3$$

$$\delta = 80 \text{ feet for the lower 23 feet; } n = \frac{23}{80} = .29$$

$$\text{Average } \delta = 5 \text{ feet for the top 10 feet; } n = \frac{10}{5} = 2$$

$$\delta = 60 \text{ feet for lower 28 feet; } n = \frac{28}{60} = .47$$

$$\text{Maximum } \delta = 10 \text{ feet for top 10 feet; } n = \frac{10}{10} = 1$$

$$\delta = 60 \text{ feet for lower 28 feet; } n = \frac{28}{60} = .47$$

A comparison of the theoretical number of droplets per square inch recovered with the actual number observed in a series of experiments conducted at the penetration test range is presented in Table 1.

Considering the heterogeneities of natural conditions and the nature of certain assumptions necessary for the solution of the theoretical equation, the theory agrees

quite favorably with the observations in the field. It shows quite definitely that insufficient droplets are produced from a 20 per cent DDT solution applied at the rate of 0.05 lb. DDT per acre to adequately penetrate the forest range studied.

In contrast to the high dense forest penetration range, three experiments were conducted on the most dense grass-type of cover to be found in any of the Tennessee

TABLE 1

Theoretical and observed penetration of insecticide through high dense forest using various particle sizes and materials applied from the Vultee spray plane 12 feet above the canopy and 50 feet above the sampling range

D_0 MICRONS	MATERIAL	APPLICATION RATE		NO. OF DROPLETS RECOVERED PER SQ. IN.						EXPECTED AVERAGE LARVAL KILL ACROSS RANGE
				Theoretical			Observed			
		Lbs. DDT/A.	drops/Sq. In.	Max.	Avg.	Min.	Max.	Avg.	Min.	
										per cent
80	30 per cent DDT in Sovacide	0.500	105.1	37.5	12.8	1.2	22	10.9	4.0	92
150	30 per cent DDT in Sovacide	0.500	63.4	22.6	7.9	0.7	13	7.1	1.0	89
95	20 per cent DDT in Sovacide	0.524	125.5	42.6	14.1	1.3	12	6.8	3.0	85
150	20 per cent DDT in Sovacide	0.524	99.6	34.2	11.6	1.1	16	8.0	4.0	87
80	20 per cent DDT in Sovacide	0.050	16.4	5.9	1.9	0.2	3	1.1	0.3	60
60	5 per cent DDT in Fuel Oil	0.070	136.2	38.6	13.0	1.2	9	3.6	1.0	57
140	5 per cent DDT in Fuel Oil	0.070	73.0	23.1	7.0	0.6	27	6.4	1.0	76

TABLE 2

Theoretical and actual penetration through low, dense, herbaceous vegetation of a 20 per cent DDT larvicide having a D_0 equal to 80 microns applied from the Vultee spray plane 25 feet above the sampling range

APPLICATION RATE		NO. OF DROPLETS RECOVERED PER SQ. IN.					
		Theoretical			Observed		
Lbs. DDT/A	drops/Sq. In.	Max.	Avg.	Min.	Max.	Avg.	Min.
.05	16.4	9.5	8.0	5.2	4.3	1.3	0.7
.09	25.9	15.0	14.2	8.9	4.3	1.7	0.7
.16	51.9	30.0	28.3	17.8	12.0	2.3	0.7

Valley reservoirs. This range was set out in solid, head-high stands of wild millet, *Echinochloa crus-galli*, in Hales Bar Reservoir. The density values (δ) for minimum, average, and maximum recovery of droplets were assumed to be 2.5, 5, and 7 feet, and n = 2, 1, and 0.7, respectively. The results of these tests for the best 100 feet of a single swath laid down by a Vultee BT-13 airplane flying 20 feet above the tops of the vegetation are tabulated in Table 2.

Here it may be observed that, although dense, the shallow depth of the vegetation as compared to that of the forest permitted the spray to penetrate much more readily. Therefore the dosages of insecticide applied could be much lower than those required for forest areas.

DIAMETER OF SPRAY DROPLETS

In summary, it appears that for the penetration of vegetation, emphasis should be placed upon particle size to insure obtaining a composite spray having a D_o in the range of 75 to 100 microns. During the course of the study some 2000 droplets

TABLE 3

Diameter class distribution of spray particles recovered above and below a forest canopy

DIAMETER CLASS, MICRONS	PERCENTAGE OF TOTAL DROPLETS RECOVERED	
	Above Canopy	Below Canopy
1– 50	54.5	81.5
50–100	32.1	14.3
100–150	9.6	2.9
150–200	2.2	1.1
200–	1.6	.2

TABLE 4

The effects of solvent density and particle size upon settling velocity

DIAMETER, MICRONS	SETTLING VELOCITY—FT./MINUTE		
	5 per cent DDT-Fuel Oil, Sp.g. 0.857	20 per cent DDT-Sovacide, Sp.g. 1.055	30 per cent DDT-Sovacide, Sp.g. 1.0905
25	3.2	4.0	4.4
50	12.7	15.5	16.5
75	29.5	35.0	37.0
100	51.0	62.0	65.0
150	114.0	140.0	146.0

were collected in the open, adjacent to the forest penetration range, and also beneath the forest canopy; these droplets were counted and measured. In the open, the D_o of all droplets collected was 130 microns with a q value of $\frac{1}{2}$. Below the canopy of the test range the D_o of droplets recovered was 90 microns with a q value of $\frac{1}{2}$. These data are presented in Table 3. This would confirm the theory in that greatest dependence for forest penetration will fall on the droplets in the 1-50 micron diameter class.

SOLVENT AND INSECTICIDE CONCENTRATION

The greater the density of the solvent the greater is the settling velocity of the spray. As droplets fall faster, the chances of impingement on vegetation by horizontal drift are less for any given spray diameter. The effect of increased solvent density is felt more in the larger diameters than in the smaller size ranges as shown in Table 4.

Perhaps of greater importance than the specific gravity of the solvent is the con-

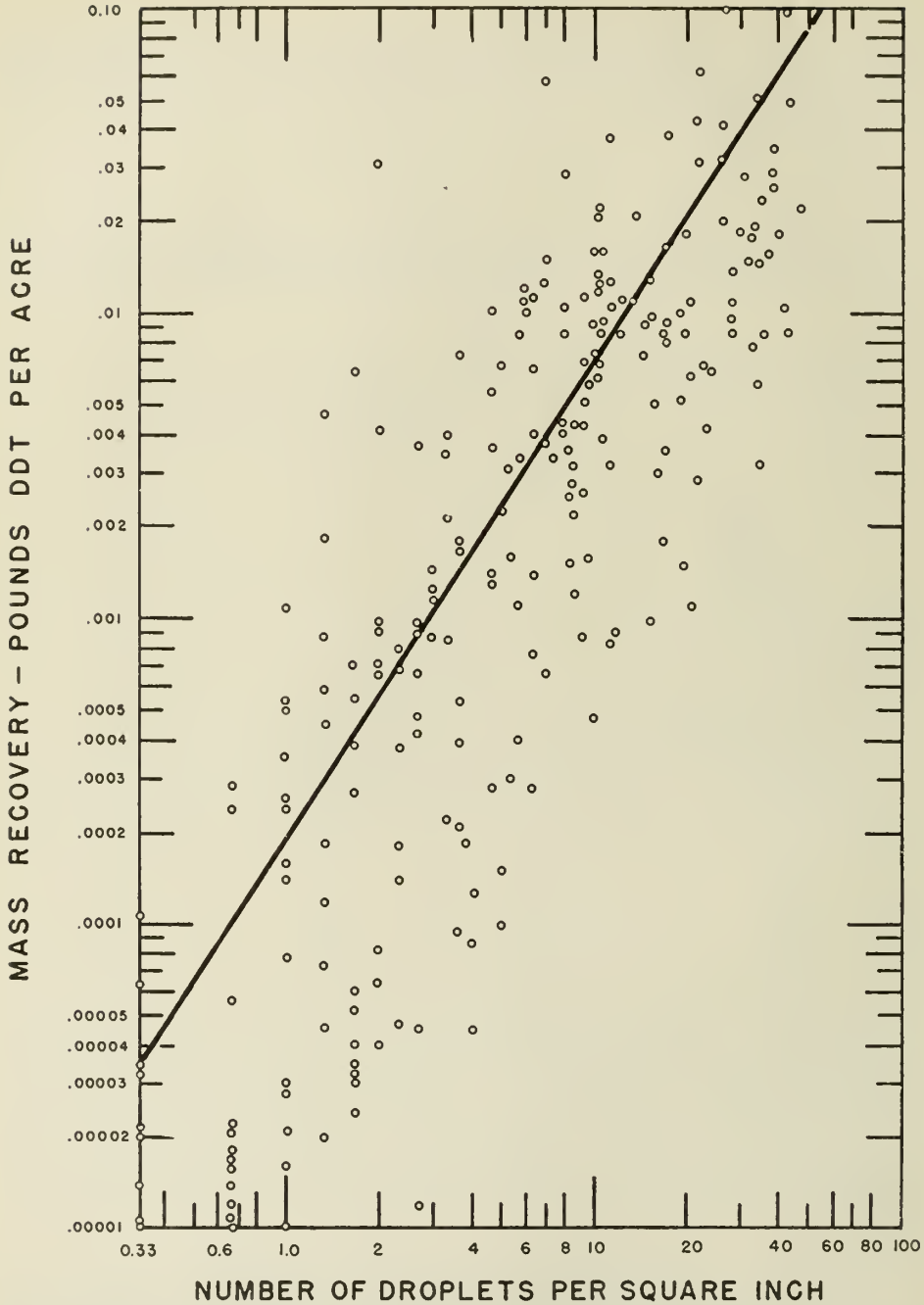

FIG. 2. Plot of data showing the mass of DDT recovered in relation to the numbers of droplets recovered from 20 per cent DDT sprays having a D_0 range of 75 to 150 microns.

centration of DDT within the droplet. While the need for adequate distribution of droplets over the breeding surface is admittedly important, the dosage of DDT recovered is directly proportional to the concentration of DDT in the droplet. Fig. 2 shows that with the present spray patterns employed, and using a 20 per cent DDT solution in Sovacide, from as few as 3 to as many as 30 droplets per sq. in. may be required to give 90 per cent or better larval mortality. The average is about 7 droplets per sq. in. for a 20 per cent DDT solution in Sovacide. By increasing the concentration of DDT to 30 per cent and disregarding the increased density of the

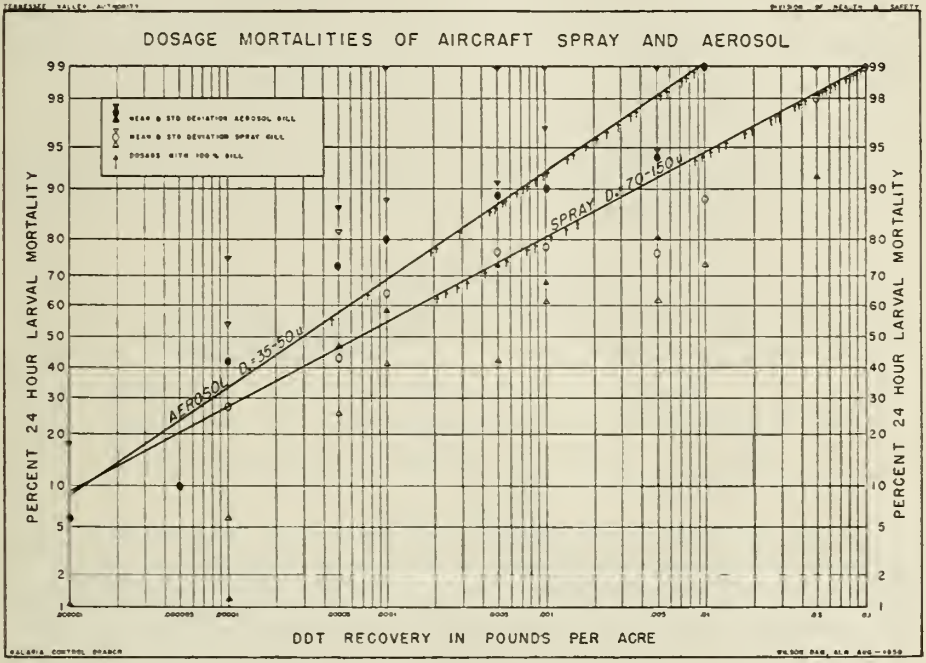

FIG. 3. Dosage-mortality experience obtained from aircraft-applied thermal aerosols of 35 to 50 microns D_o and from aircraft-applied nozzle sprays of 70 to 150 microns D_o.

solvent, the number required for 90 per cent larval kill could be expected to be reduced from an average of 7 to an average of 4 droplets per sq. in.

DDT DOSAGE MORTALITY FOR ANOPHELES QUADRIMACULATUS LARVAE

The mass of DDT in pounds per acre to be recovered for satisfactory larval mortality has been fairly well established for thermal aerosols having a D_o of 30 to 50 microns. By increasing the droplet sizes through the use of spray nozzles, the LD_{90} dose for aerosols (.0005 lb. DDT/acre) was readily recovered. Unfortunately, repeated bioassays clearly indicated that with the consequent breakup of larvicide occurring above the aerosol size range, a much higher recovery than 0.0005 lb. DDT per acre was required to consistently yield at least 90 per cent larval mortality. Fig. 3 shows the DDT dosage-mortality experience with sprays and aerosols on

early fourth stage larvae of *A. quadrimaculatus*. A profound variation in mortality is observed throughout, and although the effect is not clear-cut, it appears that a higher dosage is required for the spray fractions as opposed to the aerosols. This is indicated by the arrows which show the dosage producing 100 per cent larval mortality. Suggested values for LD_{90} and LD_{50} are given in Table 5.

It must be stated that while Table 5 shows that sprays in the 75 to 150 micron D_o range require twice the dose of DDT to equal the LD_{50} value and six times the dose to equal the LD_{90} value of aerosols, the over-all significance is not too great. From a statistical point of view, the relatively small number of mosquito larvae used in each sample indicates that repeated tests will show mortalities equal to or greater than those in the LD_{90} range, one time out of 10 trials by chance alone. In LD_{50} range, the significance is even less, with chance differences between sprays and aerosols occurring possibly three times in the course of 10 trials.

Why the aerosol should require a smaller dosage in pounds of DDT per acre is not fully understood. Possible explanations are: (1) the greater coverage afforded

TABLE 5

Recovery rates of DDT aerosols and sprays necessary to obtain varying degrees of mortality in A. quadrimaculatus *larvae*

LETHAL DOSE	REQUIRED RECOVERY LBS. DDT/ACRE	
	Aerosol	Spray
LD_{90}	0.0007	0.004
LD_{50}	0.00003	0.00007

by the more numerous aerosol droplets would make more DDT available to the larvae; (2) the increased concentration of DDT in the droplets from 20 to 25 per cent due to evaporation during thermal generation would render each droplet more efficient in contributing to larval kill, and at the same time would increase the density and insecticidal potency of the droplets in the 10 to 35 micron range; and (3) the mass of aerosol data was obtained using Velsicol NR-70 as a solvent for DDT, which in itself is a potent larvicide, and which exhibited a higher density than any solvent subsequently employed.

The present trend toward the use of nozzle sprays is not altered in any way by this seemingly anomalous finding. It is much easier to obtain consistently the higher dosage required for sprays than the lower dosage required for thermal aerosols. Further, a spray with $D_o = 80$ microns is composed of both spray and aerosol droplet diameters. This may explain the observed wide variation in recovered DDT producing satisfactory larvicidal results.

SUMMARY

Studies were conducted on the penetration of high, dense, vegetative cover by sprays applied by aircraft for the control of larvae of *A. quadrimaculatus*. Efforts were made to determine the volumes and spray spectra of insecticides required to

give satisfactory larvicidal doses on the breeding surface, and to determine, if possible, the mechanism by which spray particles penetrate vegetation.

A comparison of the resulting field data with the theory of penetration advanced by Johnstone *et al.* (1949) showed a favorable agreement. It was concluded that the best results would be obtained from spray spectra having a D_o equal to about 80 microns, and that great dependence must be placed upon particles in the 1 to 50 micron diameter class range. The volume of insecticide required to produce sufficient particles was found to depend largely upon the depth and, to a lesser degree, upon the density of the vegetative cover. Increased solvent density and solute concentration enhanced particle deposition, with a concomitant reduction in the numbers of particles required to give adequate larval control. It was found that while a deposit of only 0.0007 pound of DDT per acre was required to give an LD_{90} from applications of thermal aerosols, a deposit of 0.004 pound of DDT per acre was required to produce the same mortality from nozzle-produced sprays. However, it is pointed out that the larger deposits required from nozzle spray applications were easier to achieve than were the smaller deposits required from the application of thermal aerosols.

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RESUMEN

Se realizaron estudios sobre la penetración de riegos aplicados por aviones a densa y alta vegetación para controlar larvas de *Anopheles quadrimaculatus*. Nos esforzamos en determinar el volumen y el espectro de los riegos insecticidas necesarios para aplicar a la crianza superficial dosis larvicidas eficaces y para determinar, en lo posible, el mecanismo por el cual las partículas rociadas penetran la vegetación.

Una comparación establecida entre nuestros datos obtenidos en el campo y la teoría de penetración propuesta por Johnstone y sus colaboradores (1949) demostró un acuerdo favorable. Se concluyó que los mejores resultados se obtendrían con expectros de un Do de más o menos 80 micrones y que se debe confiar grandemente en el uso de partículas de un diámetro de 1–50 micrones. El volumen de insecticida requerido para producir suficientes partículas dependió mayormente de la profundidad y en menor grado de la densidad de la vegetación. Aumentándose la densidad del solvente y la concentración de la substancia disuelta se aumentó la deposición

de las partículas resultando en una reducción del número de partículas requeridas para efectuar un control larvicida adequado. Se demostró que aunque solo un depósito de 0.0007 de libra de DDT por acre fué necesario para causar un LD90 usándose aerosoles termales, un depósito de 0.004 de libra de DDT por acre fué necesario para producir la misma mortalidad cuando se emplearon riegos, aplicados con mangueras. Sin embargo, se noto que los depósitos mayores requeridos en las aplicaciones con mangueras se obtuvieron con más facilidad que los depósitos menores requeridos en aplicaciones con aerosoles termales.

THE SUSCEPTIBILITY OF *ANOPHELES QUADRIMACULATUS* TO DDT AFTER FIVE YEARS OF ROUTINE TREATMENT IN THE TENNESSEE RIVER VALLEY

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The first large-scale applications of DDT larvicidal and adulticidal formulations to certain impoundage areas of the Tennessee River were made during the anopheline breeding season of 1945. This initial work was so successful that DDT was the only insecticide used during the subsequent years. Thus, the year 1950 was the sixth season in which DDT was applied in certain areas. In the light of investigations carried out during 1948 and 1949 on the development of resistance by the housefly to DDT and other chlorinated hydrocarbon insecticides, and in 1949 to the development of resistance to DDT by certain species of mosquitoes (Babers, 1949), it was deemed advisable to determine whether or not resistance had developed or was in the process of development in *Anopheles quadrimaculatus* in the Tennessee River Valley. This appeared particularly important since Fay *et al.* (1949) demonstrated the development of a degree of DDT resistance in adults of a laboratory colony of *A. quadrimaculatus*. Of further importance were the implications to the malaria control program of the Tennessee Valley Authority involved in the possible presence of DDT-resistant *A. quadrimaculatus*. During the past five years the chemical control phases of this program have been based upon the efficiency of DDT both as a larvicide and as an adulticide for the control of the principal malaria vector, *A. quadrimaculatus*. Consequently, a detailed experimental program was outlined to answer the question of whether or not *A. quadrimaculatus* has developed resistance to DDT as the result of repeated applications of larvicides and of adulticides.

SUSCEPTIBILITY OF LARVAE

Data from a test using *A. quadrimaculatus* larvae from an area which had received five years of routine DDT larvicidal treatment, and from an untreated area, are presented in Table 1. The per cent mortalities given are the averages of three replications. Fifteen fourth instar larvae and 100 cc. of DDT solution were used in each replicate. The DDT was first dissolved in isopropyl alcohol, and solutions were prepared by the addition of 1 cc. of DDT-alcohol solution of appropriate concentration to one liter of tap water. The final concentration of alcohol in all solutions, including the checks, was 0.1 per cent. Abbott's formula was used to correct the observed mortalities in treated specimens for the mortalities occurring in the controls. The data would seem to indicate that the larvae from areas previously treated with DDT were less susceptible than were larvae from untreated areas; the differences are statistically insignificant. It is concluded that the routine application of DDT as a larvicide has not induced a marked differential susceptibility to that insecticide

in two naturally occurring populations of *A. quadrimaculatus* larvae in the Tennessee River Valley.

SUSCEPTIBILITY OF ADULTS

Field Laboratory Tests. Adult female *A. quadrimaculatus* were collected from their diurnal resting places in untreated and premises-treated areas of Kentucky Reservoir

TABLE 1

Per cent mortality of fourth instar A. quadrimaculatus *larvae from larvicided and untreated areas of Kentucky Reservoir following exposure to solutions of DDT for 18 hours*

SOURCE OF LARVAE	PPM OF DDT		
	0.2	0.1	0.0
Larvicided area	53.6	37.8	3.4
Untreated area	73.6	45.9	2.5

FIG. 1. View of field laboratory showing equipment and arrangement for testing adults in treated boxes.

for this series of tests. The premises in the treated area had been sprayed with residual-type DDT formulations for the sixth consecutive year prior to the initiation of these tests. All testing was carried out in a forested area under natural temperature and humidity conditions, except that shelter from direct sunlight and rain was

provided (Fig. 1). The collections were made in the morning and the tests were begun in the early afternoon. Approximately 15 adult female *A. quadrimaculatus* were used per test, and each test was run in triplicate. Tests of the two populations were carried out simultaneously. The tests were run in $7\frac{1}{4}$ x $7\frac{1}{4}$ x $4\frac{1}{2}$ inch cardboard boxes set upon framed screen wire bases. The interiors of the boxes were treated with DDT residues applied at the rate of 200 mg. per sq. ft. The deposits were aged for two weeks prior to use. The concentrate employed consisted of 30 per cent DDT, 2 per cent Triton X-100, and 68 per cent xylene, all by weight. This was thoroughly mixed with water prior to spraying. The adults were collected in glass tubes from holding cages and introduced into the test boxes through holes provided for the purpose. At the end of each exposure period the box containing the mosquitoes was placed in a "knock-out" chamber and the mosquitoes were anesthetized by exposure to CO_2 for one and a half minutes. They were then transferred to holding cages and mortality counts were made 20 hours after exposure to DDT. The control mosquitoes were handled in the same manner as the exposed mosquitoes except that the test

TABLE 2

The mean 20-hour per cent mortalities of female A. quadrimaculatus *from premises-treated and untreated areas following exposure to two-week-old deposits of DDT applied at the rate of 200 mg. per sq. ft.*

AREA SOURCE	EXPOSURE PERIOD, MINUTES	NUMBER OF REPLICATES	PER CENT MORTALITY	PROBABILITY THAT RESULTS OCCURRED BY CHANCE, (PER CENT)
Treated	10	21	18.2	
Untreated		18	27.1	16
Treated	40	21	54.8	
Untreated		18	60.5	32
Treated	90	21	86.6	
Untreated		18	93.7	0.6
Treated	Controls	19	11.8	
Untreated		18	4.2	

boxes were not treated with DDT. The controls were allowed to remain in the chambers for 90 minutes before being anesthetized. Abbott's formula was used to correct for any mortality which occurred in the unexposed controls. The final data for each exposure period were analyzed by means of the chi-square fourfold table procedure. The results of these tests are presented in Table 2.

The results obtained from these laboratory-type tests show that adult female *A. quadrimaculatus* from premises-treated and untreated areas of Kentucky Reservoir have not developed a significant differential susceptibility to residual DDT deposits. However, the data for the 10-minute and 40-minute exposure periods would seem to indicate a trend toward a decreased susceptibility in the treated population but, if present, it is not great enough to be apparent statistically. Even though the

results obtained following exposure for 90 minutes apparently were not due to chance, the difference in mortality between the two populations is much too small to be considered of practical significance.

Studies in DDT-Treated Room. Field tests to determine the comparative susceptibility of two populations of adult *A. quadrimaculatus* to routine applications of DDT residual deposits were carried out during July and August 1950. Adults for these tests were obtained from areas in which DDT premises treatment had been utilized for at least five years and from areas in which DDT had not been utilized as either a larvicide or an adulticide.

The room in which testing was done was located in a premises treatment area and was approximately 8 x 10 x 6 feet in size, slanting up to 9 feet on one side. A window and a screen door were located in opposite ends of the room. Wall and ceiling surfaces were covered with brown wrapping-paper which was treated with 5 per cent DDT emulsion applied at the rate of 200 mg. per sq. ft. on May 26. During the periods of testing, the floor was covered with cheesecloth to facilitate finding the adults which were knocked down. A screen wire trap was attached to the outside of the upper half of the window to intercept mosquitoes leaving the room.

Observations made on test mosquitoes subsequently released in the treated room included period of egress, rate of knockdown, 24-hour and 72-hour mortality, feeding habit, and general reactions of the specimens. Mosquitoes which fell to the floor and were unable to regain a resting position on the wall or ceiling were considered knocked down even though they were still capable of flight. Knocked down mosquitoes, as well as those entering the outside cage, were placed in pint ice cream cartons having screen wire ends, and were retained therein for mortality counts. An individual was recorded as being dead when no visible movement of its abdomen or appendages, including mouthparts, could be detected when it was agitated by gently shaking the holding carton. Mortalities were not tabulated beyond 72 hours, although some released mosquitoes were alive longer than 80 hours after the tests were begun. Only female mosquitoes were used in the following tests, as a preliminary test in the same room with a treated surface seven weeks old had shown that males were more susceptible than females to DDT. Total mortality of males was attained in 24 hours. Controls were used in each test, these mosquitoes being kept on the back porch of the residence next to the treated room.

Wild-caught, mostly blood-fed mosquitoes from a treated area and from an untreated area were released simultaneously in the sprayed room 8 and 11 weeks, respectively, after the application of DDT. Mosquitoes used in the 8-week test from the untreated area were reared from wild-caught mature larvae and pupae. The mosquitoes from the treated area were dyed with Rhodamine B, and those from the untreated area were unstained. The populations were subsequently separated by examination of the mosquitoes wetted with alcohol under ultra-violet light. After liberation in the room the mosquitoes were prevented from going to the window trap for a minimum period of ten minutes by means of a cheesecloth screen. This was done to increase the possibility that the mosquitoes would rest on the treated surfaces. Detailed observations on movements, knockdown, and mortalities were made, and the results are summarized in Table 3. In these tests, about 30 control

mosquitoes were used for each of the four populations, and survival at both 24 and 72 hours was 97 per cent or more in all cases. The room temperature during the period of the tests ranged from 80° to 88° F.

These results show that mosquitoes from both populations left the room in large numbers. After release the flight pattern was somewhat similar to that described by Metcalf *et al.* (1945), except that egress from the room occurred over a longer period, and as many as 15 per cent of the released mosquitoes sought the light window cloth and screen door immediately upon release, rather than the dark corners. Of the mosquitoes remaining in the room, similar knockdowns and high mortalities resulted for both populations. Of the mosquitoes collected in the escape trap, there was high survival of those from both the treated and untreated areas. In both cases, the survival of the mosquitoes which left the room was higher for the population from the

TABLE 3

Reactions of wild mosquitoes from treated and untreated areas, respectively, released simultaneously in residual sprayed room 8 and 11 weeks, respectively, after application of DDT at a rate of 200 mg. per sq. ft. applied May 26, 1950. (Mosquitoes released at about 9 A.M. and permitted to escape into window trap)

	AGE OF TREATED SURFACE			
	8 Weeks		11 Weeks	
	Treated Area	Untreated Area	Treated Area	Untreated Area
No. of female quads	133	77	118	79
Per cent leaving room to enter trap	84	45	81	78
Per cent trap mortality (72-hour)	76	83	24	40
Per cent room mortality (72-hour)	100*	100*	96	88

* Per cent knockdown over 8-hour period; similar tests with 100 per cent knockdown gave 98 per cent mortalities at 72 hours.

treated area. The maximum survival of mosquitoes entering the trap was 76 per cent for a treated source and 60 per cent for an untreated source. Metcalf (1945) and Gahan (1945) found a 95–100 per cent mortality within 24 hours among exposed mosquitoes which voluntarily left the treatment chamber. Our results indicate a considerably higher rate of survival at 24 hours for mosquitoes from the treated and untreated areas, with only a slight increase in mortality occurring between 24 and 72 hours. This high survival rate suggests that female anophelines exposed to DDT might remain in sufficient numbers in the treated areas to maintain a future population, and possibly to serve as a nucleus for the development of a DDT-resistant or tolerant strain.

In another experiment, mosquitoes from the two types of areas were released on two consecutive days at the same hour in the sprayed room 14 weeks after the application of DDT. The room temperature during the testing period ranged from 74° to 82° F. None of the released mosquitoes were permitted to escape from the treated room. In these tests, populations from the treated and untreated areas gave similar percentages of knockdown, 24-hour mortality, and 72-hour mortality (Table 4).

Limited numbers of control mosquitoes were used in each test, and 100 per cent survival was obtained during the 16.5-hour knockdown period, and extended beyond the 24-hour period of mortality. It should be noted, however, that mosquitoes from both the treated and untreated areas showed a considerable delay in knockdown, with nearly 25 per cent falling after 4.5 hours, and total knockdown being extended to almost 16.5 hours. A 16-hour total knockdown was also observed in a preliminary test in the same room with the treated surface seven weeks old. Earlier, Metcalf (1945) obtained complete knockdown in 145 minutes in an experimental room more than 3 months after treatment with DDT residual spray.

During the course of the room tests, the released mosquitoes had almost constant opportunity to feed on the workers, but in all instances no blood meals were taken,

TABLE 4

Reactions of wild mosquitoes from treated and untreated areas released on two consecutive days at the same hour in a residual-sprayed room 14 weeks after application of DDT at a rate of 200 mg. per sq. ft. applied May 26, 1950. (Mosquitoes released at about 4:30 P. M. and confined to room; 103 females from untreated area and 117 females from treated area.)

KNOCKDOWN PERIOD IN HOURS	PER CENT KNOCKDOWN		PER CENT 24-HR. MORTALITY		PER CENT 72-HR. MORTALITY		PER CENT 72-HR. SURVIVAL	
	Treated Area	Untreated Area	Treated Area	Untreated Area	Treated Area	Untreated Area	Treated Area	Untreated Area
0.0–1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0–1.5	0.9	2.9	0.9	1.0	0.9	2.9	0.0	0.0
1.5–2.0	9.4	6.8	8.5	6.8	9.4	6.8	0.0	0.0
2.0–2.5	20.5	12.6	17.9	11.6	20.5	11.6	0.0	1.0
2.5–3.0	10.3	16.5	8.5	14.6	9.4	16.5	0.9	0.0
3.0–3.5	15.4	14.6	13.7	12.6	14.5	13.6	0.9	1.0
3.5–4.0	14.5	14.6	11.1	12.6	14.5	14.6	0.0	0.0
4.0–4.5	4.3	7.8	2.6	6.8	4.3	7.8	0.0	0.0
4.5–16.5	24.8	24.2	22.2	23.3	24.8	24.2	0.0	0.0
Totals	100.1	100.0	85.4	89.3	98.3	98.0	1.8	2.0

or attempted, by *A. quadrimaculatus*. In one instance, a specimen of *A. crucians* attempted to feed shortly after release in the early evening hours. It was also observed that *A. quadrimaculatus* females from treated and untreated areas deposited about 75 eggs on the sides of the holding cages during the retention period following exposure to DDT, and prior to the determination of mortality. Under more favorable conditions, it is quite probable that a much larger number of eggs would have been laid. Fay *et al* (1949) has reported on the ability of insectary-reared *A. quadrimaculatus* to mate and oviposit following exposure to 200–400 mg. DDT per sq. ft.

Studies in Demountable Resting Stations. Supplementary information on the irritant and repellent action of DDT on *A. quadrimaculatus* was also obtained at eight of the portable resting stations, described by Snow (1949), located in treated and untreated areas of the Kentucky Reservoir. The interiors of half of the stations were treated with 5 per cent DDT emulsion applied at the rate of 200 mg. per sq. ft. on

June 2, 1950. All stations were then set up under wooded cover on the margins of selected breeding areas. Four stations were used in each area, and treated stations were alternated in line with the untreated ones. Stations were not available for use in the larvicided area until the eighth week, and collections, therefore, date from that time. Mosquitoes resting in the stations were caught in the morning, generally between 9 and 10 A.M., by two collectors, one of whom collected from the treated and the other from the untreated stations. Nineteen collections from these resting

TABLE 5

Collections from treated and untreated resting stations (traps) located in larvicided and untreated areas of Kentucky Reservoir

AGE OF TREATED SURFACE IN WEEKS	UNTREATED AREA		LARVICIDED AREA	
	Total No. of *A. quad.* Collected in Traps		Total No. of *A. quad.* Collected in Traps	
	Treated	Untreated	Treated	Untreated
5	1	82		
6	0	66		
	1	127		
7	2	112		
8	0	21	0	24
	3	16	0	11
			0	25
			0	31
9	0	18	3	30
			0	21
			0	7
11	2	8	0	1
			0	2
12	0	0	0	0
Total	9	450	3	152
Per cent	1.9	98.1	1.9	98.1

stations were made through August 23, 1950. General observations indicated that the mosquitoes were entering treated and untreated stations in approximately equal numbers. It was observed that the mosquitoes did not leave the treated stations until after they had rested upon the DDT-treated surface. Tests of the 10-week-old treated surfaces showed that four mosquitoes entering the traps were down and legless, or dead after 8 hours enclosure. In one instance, a female removed from a treated interior lived for at least three days even though she showed considerable tremor at the time of capture. These data are presented in Table 5.

Although these data show no indication of any significant resistance of *A. quadrimaculatus* to treated surfaces in the treated zone, they do support previous field tests as to the irritating and repellent action of residual DDT deposits. Metcalf *et al.* (1945) noted that several hundred adults which entered at dawn and rested in treated nail kegs near Decatur, Alabama, were all irritated and repelled within a 20-minute period, with most of them going to untreated kegs nearby. Our observations indicated, however, that 1.9 per cent remained in the treated stations at least 4 to 5 hours after entry.

Although *A. quadrimaculatus* was the most prevalent insect in the demountable traps, two other species appeared quite consistently: 101 *Culex erraticus*, 16 of which were taken in the treated stations, and 29 midges, 7 of which were found in the treated stations. Since about 25 per cent of the midges collected were in the treated traps, two series of 36 specimens were exposed for 90 minutes to DDT-treated surfaces with 100, 200, and 400 mg. per sq. ft., respectively. Total mortality occurred at all concentrations within 24 hours but no loss of legs was observed as compared with the striking loss of legs of *A. quadrimaculatus* following exposure to DDT.

SUMMARY

Comparative tests of larval populations of *Anopheles quadrimaculatus* from an untreated area, and from an area treated with DDT larvicide for five years, showed that no differential susceptibility to DDT has occurred as a result of the routine treatment. Likewise, laboratory-type tests of adult populations of the same mosquito from an untreated area, and from an area in which the premises had been treated with residual DDT deposits for at least five years, showed no significant differential susceptibility induced by the routine treatment. Comparative tests using these adult populations carried out in a room which received a routine premises treatment indicated that differences in reaction pattern have developed since DDT was first used. The mosquitoes showed a considerable delay in knockdown with nearly 25 per cent falling after 4.5 hours and total knockdown being extended to 16 hours. Among those females leaving the treated room after exposure to DDT, a fairly high rate of survival after 24 hours was obtained, with only a slight increase in mortality occurring between 24 and 72 hours. Seventy-two hours following exposure, the survival of females from the treated area was greater than that of those from the untreated area. Exposed mosquitoes were found to have laid eggs upon the sides of the holding cages.

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RESUMEN

Experimentos comparativos en poblaciones de larvas de *Anopheles quadrimaculatus* provenientes de un área virgen y de un área tratada con larvicida DDT por espacio de cinco años demostraron que no ha ocurrido una suceptibilidad diferencial como resultado del tratamiento rutinario. Igualmente, exámenes del tipo de laboratorio con poblaciones adultas del mismo mosquito provenientes de un área virgen y de un área en la cual las propiedades habían sido tratadas con depósitos de DDT residual por un espacio mínimo de cinco años no demostraron suceptibilidad diferencial considerable inducida por el tratamiento rutinario. Exámenes comparativos con estas poblaciones adultas llevados a cabo en un cuarto sometido a un tratamiento de propiedades rutinario, indicaron que se han desarrollado diferencias en el tipo de reacción desde que se aplicó el DDT por primera vez. Los mosquitos demostraron un retraso considerable en el golpe mortal con un 25 porciento aproximadamente de caídas después de 4.5 horas y el golpe mortal total llegando a 16 horas. Una considerable proporción de sobrevivientes se obtuvo después de 24 horas entre aquellas hembras que habían abandonado el cuarto después de la exposición al DDT con solo un ligero aumento en mortalidad ocurrido entre las 24 y 72 horas. Setenta y dos horas después de la exposición, la supervivencia de hembras provenientes del área tratada fué mayor que la de aquellas provenientes del área virgen. Se halló que los mosquitos expuestos habían puesto huevos en las paredes de sus jaulas.

A MALARIA RECONNAISSANCE IN THE DOMINICAN REPUBLIC[1]

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A malaria reconnaissance was conducted on four sugar estates of the West Indies Sugar Corporation in the Dominican Republic in July, August and September 1949, and in December 1949 and February 1950. This included determination of spleen and parasite indices, limited investigations of the anophelines of the region and of the potentially important anopheline breeding areas.

The region in which the investigation was conducted lies along the south coast of the island of Hispaniola to the east of the capital city, Ciudad Trujillo, extending inland from a line roughly paralleling the seacoast, but 3 to 5 kilometers from it, to the foothills of the central range of mountains. The coastal plain on which the sugar estates are located is, in effect, an extensive coral shelf, originally sea bottom. This coral formation lies close to the ground surface, and is covered in most areas with a very limited layer of soil. Underground streams originating in the foothills supply deep wells which constitute the principal water sources of the area. The ground surface in the cane fields presents numerous sink-holes common in coral and limestone formations which in many instances provide drainage of surface water during the rainy season. In other instances they are responsible for permanent or semi-permanent collections of water in and about the cane fields. Between the northern limits of the cane areas and the mountains there is a strip of relatively infertile and undeveloped land containing swamps, small ponds and numerous small lagoons, many of which hold water throughout the year. Apart from the labor population in the cane fields which is housed in the vicinity of wells, the indigenous population is collected in small scattered villages in close proximity to the lagoons, which constitute the most important source of water for man and domestic animals.

The climate is sub-tropical and characteristic of the northern Caribbean area. The temperatures are moderate and there is relatively little variation between summer and winter. Throughout the greater part of the year the northeast trade wind blows constantly from sunrise to sunset. Due to the configuration of the mountains, this is actually a sea breeze from the southeast. During the night there is usually a cool light breeze from the north. The rainy season, beginning usually in midsummer, commonly extends into late November or early December. It is characterized by frequent heavy showers particularly during the afternoon hours, with brilliant sunlight intervening. The monthly rainfall during this season in recent years has varied from approximately six to eleven or more inches of rain per month. The dry season extending

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through the winter and spring seldom presents a rainfall in excess of two inches per month. (Figure 1)

However, the rainfall distribution over the sugar estates varies markedly within comparatively short distances. In general it is relatively low along the coast, increasing progressively to a maximum in the northern areas near the foothills. During the past seven years or more the rain fall in all areas has been significantly below the average of the previous decade. This in turn has apparently been accompanied by a decrease in the incidence of clinical malaria.

The population of the area covered by the reconnaissance is scattered and much

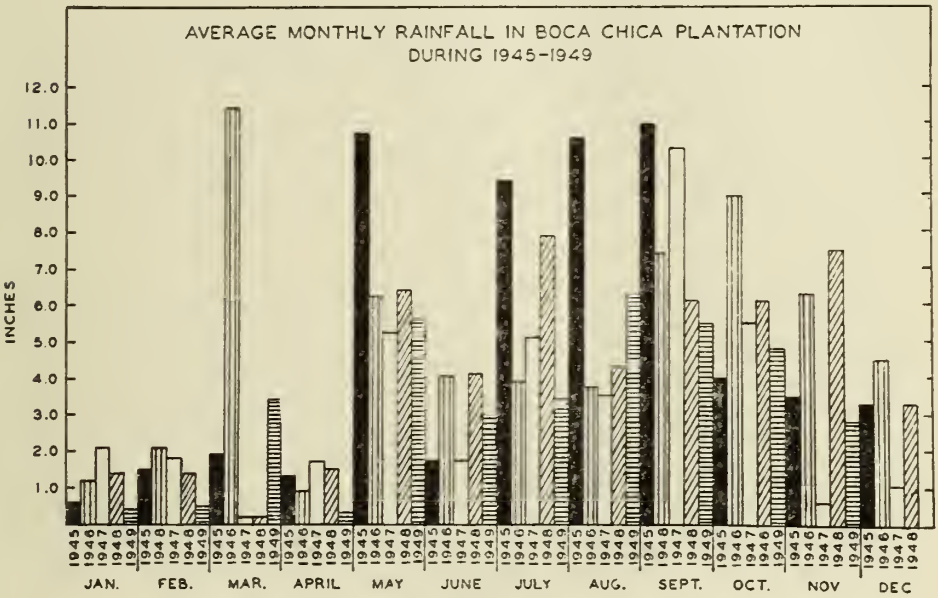

FIG. 1

of it apparently migratory, in the sense that individual families move to new sites in the same general region as the productivity of their gardens diminishes. The seasonal nature of work on the sugar estates contributes further to this population movement. No accurate census figures are available.

The people are of mixed Spanish origin. Since the original colonization of the eastern half of the island by the Spaniards, a certain admixture of Negro blood has occurred especially among the rural population, partly the result of invasions of the country by Haiti during the colonial period and partly the result of importation of Haitian labor and sporadic migration across the border between the two countries. The net result, however, is a people who are much more Spanish than Negro in appearance and culture.

No malaria survey in the Dominican Republic has been published previously. Consequently it is impossible to contrast the findings in this investigation with those of others. It must be emphasized that the data and the interpretations of them are

applicable only to the area in which these studies were conducted. It is recognized that both the endemicity and the epidemiology of malaria differ in other areas of the Republic. For example, it is known that the development of extensive areas of wet rice culture in certain regions has been associated with a marked change in magnitude of the local malaria problem. In other areas the culture of sugar cane under irrigation has led to change in local endemicity. The limited information at present available indicates that only three anophelines have been identified in the Dominican Republic, *Anopheles albimanus*, *A. grabhamii*, and *A. vestitipennis*. Of these, *A. albimanus* alone is recognized as a natural vector (Carr, 1950).

The findings of an extensive malaria reconnaissance in the Republic of Haiti in the western portion of the island of Hispaniola abutting on the western boundary of the Dominican Republic constitute the only data for comparative purposes (Paul and Bellerive, 1947). Splenomegaly was noted in 18.4 per cent of the children examined, and the adjusted parasite rate was 31 per cent. *Anopheles albimanus* was found to be the important vector. *Anopheles grabhamii* and *A. vestitipennis* were likewise identified. *Plasmodium falciparum* was responsible for 86.6 per cent of the total infections, *P. malariae* for 8.9 per cent, and *P vivax* for only 1.9 per cent.

METHODS

The investigation was conducted at two seasons of the year to obtain information concerning the maximum incidence of malaria in the labor population, and to determine the season of maximum transmission. The first group of observations in the summer of 1949 were made at the beginning of the rainy season. The second group of observations, made in the late autumn and the winter of 1950, coincided with the start of the dry season. A total of 4,656 children were examined for splenic enlargement. Thick and thin blood films were taken from 4,592 of these individuals. The films were stained with Giemsa immediately upon return to the field laboratory each day, and they were examined subsequently as rapidly as time permitted.

Surveys and Results

BOCA CHICA ESTATE: The Boca Chica estate was studied in the summer of 1949 and again in December of that year. In both instances the entire property was covered. The sugar mill and the main Batey (i.e. the headquarters area) of this estate are situated on the coast where the maximum effect of the trade winds is felt. Immediately adjacent to the Batey are two small villages from which much of the mill and railroad labor is drawn. The cane fields lie some three kilometers inland and are separated from the Batey by rough rocky country covered by scrub forest growth in which there is little or no water, and to all intents and purposes no harborage for anophelines.

In August 383 children were examined on the Boca Chica Batey. Five of these presented demonstrable splenomegaly, an index of 1.3. (Table 1) In December, only two instances of enlargement of the spleen were found among 234 children examined, an index of 0.8.

The villages in the cane areas, however, gave significantly higher rates. In August, splenomegaly was noted in 58 of 757 children examined, an index of 7.6, while in

December enlargement of the spleen was noted in 45 of 455 children examined, an index of 9.8. At each of the two seasons the areas of Mamey, Switch 17 and Cayacoa yielded the highest rates. All three of these areas lie along the northern limits of the property and within easy anopheline flight range of swamps and lagoons in which larvae of *A. albimanus* were found. Immediately adjacent to the village of Mamey a small borrow pit along the railroad was found to contain many anopheline larvae.

Blood films from 598 children examined at the main Batey in August and December were all negative. (Table 2)

TABLE 1

Spleen indices, Boca Chica plantation, August and December, 1949

BOCA CHICA PLANTATION	DATE	0–1 YR.		2–4 YRS.		5–9 YRS.		10–14 YRS.		15 YRS.		TOTAL		SPLEEN INDICES
		No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	
Boca Chica Batey	7-26 8-3	62	1	89	3	135	0	86	1	11	0	383	5	1.3
Hato Viejo	8-4	7	1	14	3	27	1	31	1	3	0	82	6	7.3
Mamey	8-8	10	0	15	4	30	9	28	2	4	0	87	15	17.2
Gautier	8-12	13	0	28	1	40	0	34	1	3	0	118	2	1.7
Switch 17	8-19	25	4	25	1	57	4	49	3	13	0	169	12	7.1
Cayacoa	8-31	41	3	51	3	69	8	53	7	4	0	218	21	9.6
Jube	8-13	8	0	22	0	33	2	19	0	1	0	83	2	2.4
August—Boca Chica villages Totals												757	58	7.6
Boca Chica Batey	12-5	28	0	31	0	100	1	71	1	4	0	234	2	0.8
Hato Viejo	12-8	4	0	6	1	28	2	22	1	0	0	60	4	6.7
Mamey	12-7	3	0	3	0	27	10	20	6	0	0	53	16	30.2
Gautier	12-8	12	0	21	0	27	1	23	0	0	0	83	1	1.2
Switch	12-11	17	2	21	3	44	4	30	3	3	0	105	12	11.4
Cayacoa	12-12	13	0	19	1	28	4	25	6	5	0	90	11	12.2
Jube	12-7	9	0	19	0	25	0	11	1	0	0	64	1	1.6
December—Boca Chica villages Totals												455	45	9.8
Boca Chica plantation consolidated Totals												1829	110	6.0

During the month of August 743 children in the cane field villages were examined. Positive blood films were obtained from 37, a parasite index of 4.9. In December films were taken from 448 children, 31 of whom had demonstrable parasitemia, an index of 6.9. The three areas where the highest incidence of splenomegaly was found, Mamey, Switch 17 and Cayacoa, likewise yielded the greatest number of positive blood films.

CONSUELO ESTATE: The Consuelo Batey was studied in August and December of 1949 and the villages in the cane fields in February of 1950. The Batey of this estate is situated some twelve kilometers inland from the coast in immediate proximity to the cane fields. It lies on high ground which is well drained, about two

kilometers from one of the two main branches of the Higuamo River. There are no swamps or lagoons in the immediate vicinity, and while *A. albimanus* larvae in considerable numbers were found at various points along the grassy margins of the river, the direction of the prevailing wind confers some definite protection.

A total of 286 children on this Batey were examined for splenomegaly in August and December. Only three instances of splenic enlargement were found in both surveys, an index of 1.0. (Table 3)

TABLE 2

Parasite indices, Boca Chica plantation, August and December, 1949

BOCA CHICA PLANTATION	DATE OF SURVEY 1949	P. FALCIPARUM		P. VIVAX		P. MALARIAE		TOTAL EXAMINED	TOTAL POSITIVE*	PER CENT POSITIVE
		No.	Per cent Pos.	No.	Per cent Pos.	No.	Per cent Pos.			
Boca Chica Batey..	7-26	0	0	0	0	0	0	367	0	0
	12-5	0	0	0	0	0	0	231	0	0
Totals		0	0	0		0		598	0	0
Hato Viejo.....	8-4	0	0	1	33.3	2	66.6	83	3	3.6
Mamey	8-8	12	80.0	0	0	6	40.0	85	15	17.6
Gautier.....	8-12	1	20	0	0	4	80	116	5	4.3
Switch 17.....	8-19	6	85.7	0	0	1	14.3	160	7	4.3
Cayacoa.....	8-31	6	85.7	0	0	1	14.3	216	7	3.2
Jube.....	8-13	0	0	0	0	0	0	83	0	0
Totals		25		1		14		743	37	4.9
Hato Viejo.....	12-8	0	0	0	0	1	100	60	1	1.6
Mamey.....	12-7	1	33.3	0	0	2	66.6	53	3	5.6
Gautier.....	12-8	2	100	0	0	0	0	83	2	2.4
Switch 17.....	12-11	14	82.4	0	0	3	17.6	102	17	16.6
Cayacoa.....	12-12	7	100	0	0	0	0	86	7	8.1
Jube.....	12-7	1	100	0	0	0	0	64	1	1.5
Totals		25		0		6		448	31	6.9
Boca Chica plantation consolidated Totals								1789	68	3.8

* Discrepancies between "Total Positive" and sum of individual species found is due to double infections.

In the cane field villages of this estate 1,014 children were examined in February 1950. Sixty instances of splenomegaly were found, giving a spleen index of 5.9. The highest index, 9.9 at the village of La Plaza, is considerably lower than the figures obtained for the three areas of highest endemicity on the Boca Chica property. This difference is readily accounted for by the relative lack of suitable anopheline breeding areas in close proximity to the villages.

Blood films were taken from 280 children on the Consuelo Batey in August and December of 1949. Only three positives were found, a parasite index of 1.0. (Table 4)

TABLE 3

Spleen indices, Consuelo plantation, August, December, 1949 and February, 1950

CONSUELO* PLANTATION	DATE	0–1 YR.		2–4 YRS.		5–9 YRS.		10–14 YRS.		15 YRS.		TOTAL		SPLEEN INDEX
		No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	
	1949													
Consuelo Batey..	8-25	23	0	42	2	67	0	98	0	3	0	233	2	0.9
	12-17	1	0	3	1	13	0	32	0	4	0	53	1	1.9
	1950													
Santo Domingo...	2-2	13	0	23	0	43	2	55	4	9	1	143	7	4.9
Vasca.....	2-3	7	2	10	1	21	0	25	2	0	0	63	5	7.9
Margarita.....	2-3	7	0	14	1	23	0	11	0	0	0	55	1	1.8
AB-4.....	2-3	17	0	18	0	17	1	11	1	0	0	63	2	3.2
Jalonga.....	2-4	11	2	11	0	15	1	14	1	0	0	51	4	7.8
Chicharones.....	2-4	24	0	28	2	41	1	40	3	4	0	137	6	4.4
Platanitos.....	2-4	13	0	19	1	19	0	19	0	0	0	70	1	1.4
La Plaza.....	2-6	42	1	73	4	97	13	78	9	2	2	292	29	9.9
Monte Coca.....	2-6	17	0	30	2	41	2	48	1	4	0	140	5	3.6
Consuelo plantation consolidated Totals												1300	63	4.8

TABLE 4

Parasite indices, Consuelo plantation, August and December, 1949; February, 1950

CONSUELO PLANTATION	DATE OF SURVEY	P. FALCIPARUM		P. VIVAX		P. MALARIAE		TOTAL EXAMINED	TOTAL POSITIVE*	PER CENT POSITIVE
		No.	Per cent Pos.	No.	Per cent Pos.	No.	Per cent Pos.			
	1949									
Consuelo Batey.....	8-25	2	100	0	0	0	0	231	2	0.8
	12-17	1	100	0	0	0	0	49	1	2.0
Totals		3		0		0		280	3	1.0
	1950									
Santo Domingo.....	2-2	6	85.7	0	0	1	14.3	137	7	5.1
Vasca.....	2-3	4	100	0	0	0	0	63	4	6.4
Margarita.....	2-3	4	100	1	25	0	0	55	4	7.2
AB-4.....	2-3	0	0	0	0	0	0	63	0	0
Jalonga.....	2-4	3	75.0	1	25.0	1	25.0	51	4	7.8
Chicharones.....	2-4	5	100	0	0	0	0	137	5	3.6
Platanitos.....	2-4	2	100	0	0	0	0	70	2	2.8
La Plaza.....	2-6	26	83.9	0	0	5	16.1	292	31	10.6
Monte Coca.....	2-6	6	85.7	0	0	1	14.3	138	7	5.1
Totals		56		2		8		1006	64	6.3
Consuelo plantation consolidated Totals								1286	67	5.2

* Discrepancies between "Total Positive" and sum of the individual species found is due to double infections.

In February, films were taken from 1,006 children in the villages in the cane fields. Parasitemia was demonstrated in 64, a parasite index of 6.3. With one exception the indices on this estate ranged from 2.8 to 7.8. La Plaza, however, where a spleen index of 9.9 was found, yielded 31 positive blood films among the 292 children examined.

LAS PAJAS ESTATE: The Las Pajas Batey was studied in August and December of 1949, and the villages in February 1950. The Batey of this estate is considerably smaller than those of the other properties. It lies about five kilometers further inland than Consuelo, and is situated among low hills in the midst of extensive cane fields. There are no swamps or lagoons in the immediate vicinity, and the Batey itself is well drained.

Only three instances of splenomegaly were found among the 325 children examined on this Batey, a spleen index of 0.9. (Table 5)

TABLE 5

Spleen indices, Las Pajas plantation, August, December 1949 and February 1950

LAS PAJAS PLANTATION	DATE	0–1 YR.		2–4 YRS.		5–9 YRS.		10–14 YRS.		15 YRS.		TOTAL		SPLEEN INDICES
		No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	
	1949													
Las Pajas Batey	8-30	10	0	54	0	87	1	50	0	0	0	201	1	0.5
	12-16	12	2	21	0	57	0	34	0	0	0	124	2	1.6
	1950													
Jiminez	2-7	2	0	8	0	25	2	17	1	3	1	55	4	7.3
Hoyon	2-8	6	1	16	1	16	6	10	8	0	0	48	16	33.3
Anita	2-8	7	1	22	4	12	7	24	6	2	1	67	19	28.4
Altagracia	2-8	2	0	14	0	18	2	11	1	1	0	46	3	6.5
Tabacones	2-8	3	0	5	0	6	1	4	0	0	0	18	1	5.6
Las Pajas plantation consolidated Totals												559	46	8.1

The cane fields of this estate extend north to the foothills of the mountains. Several small streams, tributary to the Higuamo River, flow through the cane areas. A total of 234 children in five villages were examined among whom 43 instances of splenic enlargement were found, a spleen index of 18.3. Two of the villages, however, Hoyon and Anita, gave spleen indices of 33.3 and 28.4 respectively. Each of these two small communities is situated in close proximity to a slow moving stream, from both of which larvae of *A. albimanus* and *A. grabhamii* were obtained.

Blood films were taken from 201 children on the Batey in August. Parasitemia was demonstrated in two, a parasite index of 0.9. In December additional films were taken from 124 children among whom four positives were found, an index of 3.2. (Table 6)

In February 229 children in the villages were examined. Fifty-five positive blood films were obtained, a parasite index of 24.0. The two villages of Hoyon and Anita gave indices of 29.1 and 41.7 respectively. The two other villages from which positive blood films were obtained yielded indices of 13.3 and 13.7 respectively.

QUISQUEYA ESTATE: The Quisqueya Batey was studied in September and December of 1949, and the villages in February 1950. The Batey lies about twelve

TABLE 6

Parasite indices, Las Pajas plantation, August and December, 1949; February, 1950

LAS PAJAS PLANTATION	DATE OF SURVEY	P. FALCIPARUM		P. VIVAX		MALARIAE		TOTAL EXAMINED	TOTAL POSITIVE*	PER CENT POSITIVE
		No.	Per cent Pos.	No.	Per cent Pos.	No.	Per cent P.			
	1949									
Las Pajas Batey....	8-30	2	100	0	0	0	0	201	2	0.9
	12-16	4	100	0	0	0	0	124	4	3.2
Totals.....		6		0		0		325	6	1.8
	1950									
Jiminez.....	2-7	7	100	0	0	0	0	51	7	13.7
Hoyon.....	2-8	5	35.7	1	7.1	8	57.2	48	14	29.1
Anita.....	2-8	28	100	0	0	1	3.5	67	28	41.7
Altagracia.....	2-8	4	66.7	0	0	2	33.3	45	6	13.3
Tabacones.....	2-8	0	0	0	0	0	0	18	0	0
Totals.....		44		1		11		229	55	24.0
Las Pajas plantation consolidated Totals.....								554	61	11.0

* Discrepancies between "Total Positive" and sum of individual species found is due to double infections.

TABLE 7

Spleen indices, Quisqueya plantation, September, December 1949 and February, 1950

QUISQUEYA PLANTATION	DATE	0-1 YR.		2-4 YRS.		5-9 YRS.		10-14 YRS.		15 YRS.		TOTAL		SPLEEN INDICES
		No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	
	1949													
Quisqueya Batey..	9-24	12	0	58	0	107	1	66	0	0	0	243	1	0.4
	12-15	5	0	18	0	83	2	72	0	0	0	178	2	1.2
	1950													
Consuelita.....	2-10	17	0	16	1	39	1	29	4	0	0	101	6	5.9
Ulloa.....	2-10	9	0	14	0	24	2	28	2	0	0	75	4	5.3
Canutillo.....	2-10	1	0	9	0	15	0	19	0	0	0	44	0	0
Moruna.....	2-10	8	1	18	2	33	0	15	2	1	0	75	5	6.7
Naranja.....	2-11	13	1	12	2	19	1	12	3	0	0	56	7	12.5
Estrella.....	2-11	10	0	21	1	24	1	33	3	4	1	92	6	6.5
Caimito.....	2-11	13	0	25	0	29	2	37	3	0	0	104	5	4.8
Quisqueya plantation consolidated Totals.....												968	36	3.6

kilometers inland from the seacoast in the center of an extensive area of cane cultivation. There are no swamps or lagoons in the immediate neighborhood. A main

branch of the Higuamo River, however, lies about one kilometer to the east, and the Batey therefore is within the theoretical anopheline flight range.

Of 421 children examined on the Batey only 3 instances of splenomegaly were encountered, a spleen index of 0.7. (Table 7)

The cane fields of the Quisqueya estate likewise extend to the north in proximity to the foothills where the heaviest rainfall occurs. Numerous streams run through the cane fields, ultimately joining the Higuamo River. A total of 547 children in the villages in the fields were examined. Splenomegaly was found in 33, a spleen index of 6.0. The highest index found on this estate was 12.5, at Naranja.

TABLE 8

Parasite indices, Quisqueya plantation, September and December, 1949; February, 1950

QUISQUEYA PLANTATION	DATE OF SURVEY	P. FALCIPARUM		P. VIVAX		P. MALARIAE		TOTAL EXAMINED	TOTAL POSITIVE*	PER CENT POSITIVE
		No.	Per cent Pos.	No.	Per cent Pos.	No.	Per cent Pos.			
	1949									
Quisqueya Batey....	9-24	0	0	0	0	0	0	243	0	0
	12-15	2	100	0	0	0	0	178	2	1.1
Totals.....		2						421	2	0.4
	1950									
Consuelito.....	2-10	10	76.3	0	0	3	23.7	101	13	12.8
Ulloa.....	2-10	5	100	0	0	0	0	75	5	6.6
Canutillo.....	2-10	4	100	0	0	0	0	44	4	9.1
Moruna.....	2-10	10	100	0	0	0	0	74	10	13.5
Naranja.....	2-11	8	80.0	2	20.0	2	20.0	56	10	17.8
Estrella.....	2-11	12	85.7	3	21.4	2	14.2	88	14	15.9
Caimito.....	2-11	7	87.5	4	50.0	0	0	104	8	7.6
Totals.....		56		9		7		542	64	11.8
Quisqueya plantation consolidated Totals.....								963	66	6.8

* Discrepancies between "Total Positive" and sum of individual species found is due to double infection.

Blood films were taken from 421 children on the Quisqueya Batey. Only two instances of parasitemia were found. (Table 8) In the villages 542 children were examined, 64 of whom gave positive films, a parasite index of 11.8. The highest value found, 17.8, was at the village of Naranja, which also had the highest spleen rate.

SPECIES OF PLASMODIA: In all, blood films were taken from a total of 4,592 children. Of these, 262 were positive, a rate of 5.7 per cent. *Plasmodium falciparum* was present in 216 (82.4 per cent); *P. malariae* in 46 (17.5 per cent); and *P. vivax* in only 13 (4.9 per cent). (Table 9)

SEASONAL VARIATIONS IN SPLEEN AND PARASITE INDICES: When the observations made in July, August, and September 1949 are compared with those of December 1949 and February 1950, definite differences are seen. (Table 10) Al-

though it was impossible to study all of the areas both during the summer and winter periods, where studies were carried out in the two seasons both the spleen and the parasite indices were higher in December and February.

TABLE 9

Species of Plasmodia

AREA	TOTAL EXAMINED	TOTAL POSITIVE	P. FALCIPARUM		P. VIVAX		P. MALARIAE	
			No.	Per cent Pos.	No.	Per cent Pos.	No.	Per cent Pos.
Boca Chica Batey	598	0	0		0		0	
Hato Viejo	143	4	0		1		3	
Mamey	138	18	12		0		8	
Gautier	199	7	3		0		4	
Switch-17	262	24	20		0		4	
Cayacoa	302	14	13		0		1	
Jube	147	1	1		0		0	
Consuelo Batey	280	3	3		0		0	
Santo Domingo	137	7	6		0		1	
Vasca	63	4	4		0		0	
Margarita	55	4	4		1		0	
AB-4	63	0	0		0		0	
Jalonga	51	4	3		1		1	
Chicharones	137	5	5		0		0	
Platanitos	70	2	2		0		0	
La Plaza	292	31	26		0		5	
Monte Coca	138	7	6		0		1	
Las Pajas Batey	325	6	6		0		0	
Jimenez	51	7	7		0		0	
Hoyon	48	14	5		1		8	
Anita	67	28	28		0		1	
Altagracia	45	6	4		0		2	
Tobacones	18	0	0		0		0	
Quisqueya Batey	421	2	2		0		0	
Consuelito	101	13	10		0		3	
Ulloa	75	5	5		0		0	
Canutillo	44	4	4		0		0	
Moruna	74	10	10		0		0	
Naranja	56	10	8		2		2	
Estrella	88	14	12		3		2	
Caimito	104	8	7		4		0	
Totals	4592	262	216	82.4	13	4.9	46	17.5

In the July and September study, a spleen index of 3.6 was obtained from examination of 1817 children, with a parasite index of 2.3 from 1785 of these children. In the December and February investigations, a spleen index of 6.6 was found in the

examination of 2839 children. Blood films from 2807 of these gave a parasite index of 7.8.

VECTOR STUDIES: Only very limited entomological studies were practicable in the short time available. It was not possible to attempt the collection of adult anophelines, and investigations were limited to the examination of potential breeding areas within reasonable distances of the Bateys and the villages studied. Larvae were collected wherever found and brought back to the field laboratory at Boca Chica for identification or rearing to the fourth instar for identification. A few adults were

TABLE 10

Spleen and parasite indices seasonal variations

AREA	SPLENOMEGALY			PARASITEMIA		
	Number examined	Splenomegaly	Spleen indices	Number examined	Positive	Per cent positive
July–September, 1949						
Boca Chica Batey	383	5	1.3	367	0	0
Boca Chica Villages	757	58	7.6	743	37	4.9
Consuelo Batey	233	2	0.9	231	2	.8
Las Pajas Batey	201	1	0.5	201	2	0.9
Quisqueya Batey	243	1	0.4	243	0	0
Totals	1817	67	3.6	1785	41	2.3
December, 1949–February, 1950						
Boca Chica Batey	234	2	0.8	231	0	0
Boca Chica Villages	455	45	9.9	448	31	6.9
Consuelo Batey	53	1	1.9	49	1	2.0
Consuelo Villages	1014	60	5.9	1006	64	6.3
Las Pajas Batey	124	2	1.6	124	4	3.2
Las Pajas Villages	234	43	18.3	229	55	24.0
Quisqueya Batey	178	2	1.1	178	2	1.1
Quisqueya Villages	547	33	6.0	542	64	11.8
Totals	2839	188	6.6	2807	221	7.8

reared from larvae to confirm species identification, and *A. grabhamii* was definitely identified.

Only two species of anophelines, *A. albimanus* and *A. grabhamii*, were found. The larvae of the former were invariably in sun lit water, such as the grassy margins of lagoons, borrow pits, in the aquatic growth along the margins of the Higuamo River and its tributaries, and in quiet stretches of small streams exposed to full sunlight. In most of the areas examined, larvae were not present in great numbers, and many apparently favorable collections of water yielded negative results to dipping. In no instance were larvae recovered from cattle tracks or ruts in roads, as is common in some of the hyperendemic areas of the Caribbean region.

Larvae of *A. grabhamii* were found only in two streams near the foothills in the

vicinity of the villages of Hoyon and Anita on the Las Pajas estate. They were taken principally from deeply shaded areas.

DISCUSSION

Both the spleen indices and the parasite indices point to a low malaria endemicity in the general area investigated. Within this region, however, isolated areas of relatively high incidence were found, notably the two villages of Hoyon and Anita, where spleen and parasite indices approximated 30 to 40 per cent. These villages lie well toward the northern limits of the sugar properties, close to the foothills, near streams, and in an area of maximal annual rainfall. Without exception the highest rates were obtained in the northern portion of the properties. As the seacoast was approached both the spleen and parasite indices dropped close to zero.

The comparative findings at the two seasons of the year indicate that the period of maximum transmission of malaria corresponds with the autumn months and the latter part of the rainy season.

It is of interest that the relative proportions of the three species of plasmodia found quite closely approximates that observed by Paul and Bellerive (1947) in Haiti. The relatively high incidence of *P. malariae* in contrast to *P. vivax* may represent a purely seasonal variation. Our data as yet are insufficient to determine this. Another factor, however, requires consideration. Rainfall data kept by the sugar estates has shown a marked decrease in annual rainfall totals for nearly a decade. This inevitably has had an effect upon anopheline distribution. The local geographical variation in splenomegaly and parasitemia together with the relatively high incidence of *P. malariae* infections may indicate a decreasing malaria endemicity.

Further this same distribution of splenomegaly and parasitemia strongly suggests that considerable numbers of the population on the sugar estates have lost their premunition and that there is in fact a relatively large non-immune population in the areas more closely adjacent to the seacoast. A change in the weather cycle with a series of years of greater and more evenly distributed rainfall might be accompanied by increasing endemicity which might reach epidemic proportions.

CONCLUSIONS

1. The data of a malaria reconnaissance in the southeast portion of the Dominican Republic are presented.
2. In the summer months at the beginning of the rainy season a spleen index of 3.6 was obtained from 1817 children examined.
3. In the months of December and February, at the end of the rainy season, examination of 2839 children gave a spleen index of 6.6.
4. Parasite indices at the two seasons based upon blood films from 1785 and 2807 children respectively, were 2.3 and 7.8.
5. *Plasmodium falciparum* was found in 82.4 per cent, *P. malariae* in 17.5 per cent, and *P. vivax* in 4.9 per cent of the positive blood films.
6. Two species of anophelines were found, *Anopheles albimanus* and *A. grabhamii*.
7. The endemicity of malaria in the region studied appears to be at a minimum

near the seacoast. It rises progressively to a maximum near the foothills of the mountains where the highest annual rainfall occurs.

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CONCLUSIONES

1. Se presentan los datos relativos a un reconocimiento de malaria en la porción sureste de la República Dominicana.

2. En los meses de verano al empezar la estación lluviosa se obtuvo en 1,817 niños examinados un índice esplénico de 3.6.

3. En los meses de diciembre y febrero, al terminar la estación lluviosa, se obtuvo en 2,839 niños examinados un índice esplénico de 6.6.

4. En las dos estaciones se registraron índices parasíticos de 2.3 y 7.8 mediante el examen de laminillas de sangre de 1,785 y 2,807 niños respectivamente.

5. *Plasmodium falciparum* se encontró en 82.4 porciento, *P. malariae* en 17.5 porciento y *P. vivax* en 4.9 porciento de las laminillas de sangre positivas.

6. Dos especies de *Anófeles* se encontraron, *Anófeles albimanus* y *A. grabhamii*.

7. La endemicidad de malaria en la región estudiada parece ser mímima cerca de la costa. Se aumenta progresivamente a un máximum cerca del pie de las montañas donde la cantidad de lluvia anual es más alta.

OBSERVATIONS ON THE NATURAL OCCURRENCE OF *PLASMODIUM FLORIDENSE*, A SAURIAN MALARIA PARASITE, IN *SCELOPORUS UNDULATUS UNDULATUS*

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As part of a program to study the infectivity of local sylvan malarias to *Anopheles* mosquitoes, surveys were made of blood parasites of reptiles in southwestern Georgia. Infections of *Plasmodium floridense* Thompson and Huff (1944) were found in two species of lizards, the common chameleon, *Anolis carolinensis*, and the southern fence lizard, *Sceloporus undulatus undulatus*. An ecological study of *S. u. undulatus* in the area had shown that individuals of this species, particularly the females, move over a limited geographic range. Only occasionally will females move out of an area of 30 feet radius. Individuals appear to inhabit specific logs or trees and may be observed on or in the vicinity of them repeatedly (Crenshaw, unpublished). This habit of the lizards offered the possibility of observing malaria infection rates in a population of these animals and of following the course of individual infections under natural conditions.

METHODS

A two acre laurel oak hammock adjacent to a limesink pond in Baker County, Georgia was selected for the study. The plot had sparse undergrowth and was shaded by several large oak and a few gum, pine, and other trees. Field observations were made from July 6, 1949 through May 12, 1950. Visits were made to the area regularly, never less frequently than once a week, except for three instances during the winter. During the 44 weeks of the study the area was visited 52 times. Systematic searches were made for lizards which could ordinarily be captured without difficulty. A snare, on a cane pole, was used to take specimens too high to reach unaided. Blood films were made from each specimen captured. Lizards were marked for subsequent identification by clipping a combination of toes. Each lizard was designated by letter and blood films were marked in the field. The animals were measured and estimates were made of their age. Individuals were designated as juvenile or adult. A juvenile was considered to be a hatchling of the current season. Hatching in this area usually begins about the middle of June and is complete by the last of September.

Blood films were stained with Giemsa. Parasites were enumerated and results expressed in terms of the number of parasites per 10,000 red blood cells. Ordinarily it was possible to obtain only a very small amount of blood from a lizard without severe injury to the specimen. As a result the parasite density had to be determined from counting a relatively small number of blood cells. When the parasite count was

above 500 per 10,000 red blood cells, it was usually possible to keep the probable error under 15 per cent; counts with a probable error greater than 20 per cent were designated only as positive.

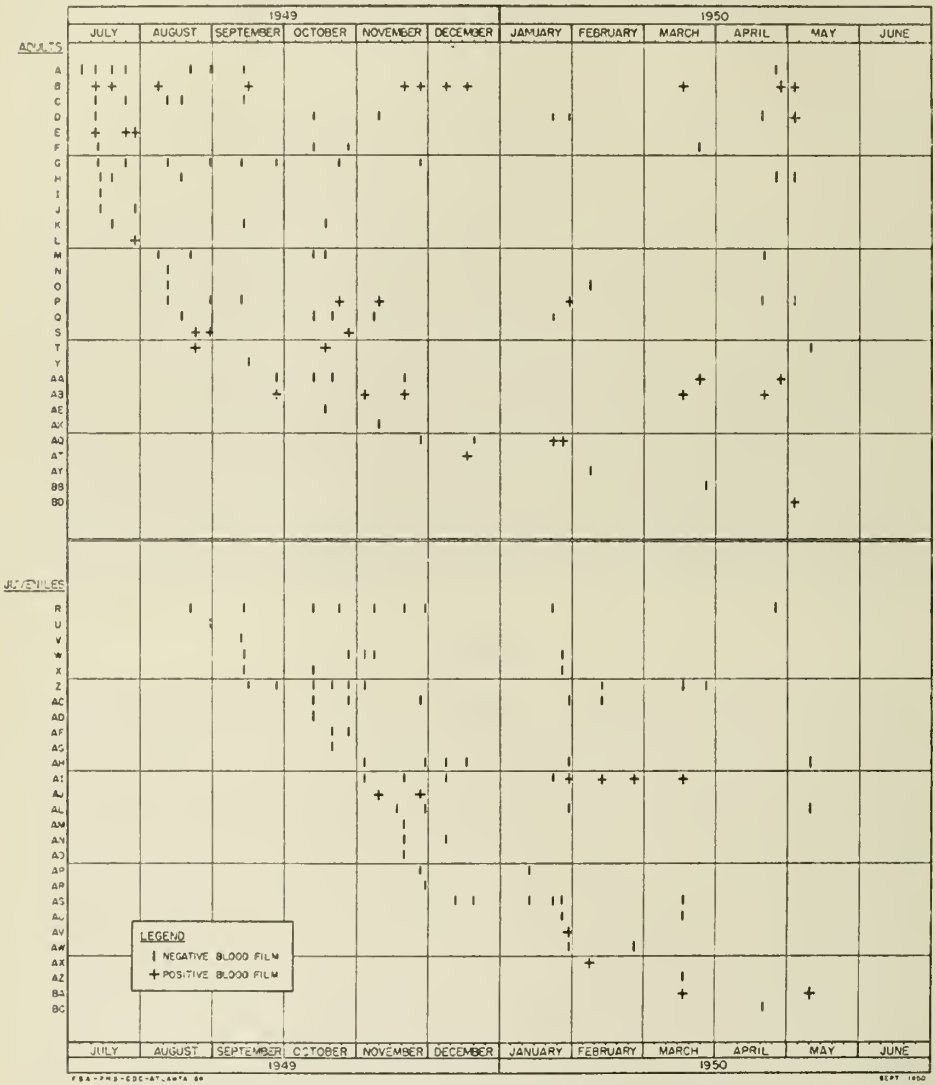

FIG. 1. Dates of capture and results of blood film examinations of *Sceloporus u. undulatus* in the intensive study area.

Attempts were made frequently to collect lizards outside of the intensive observation area to provide additional information on seasonal infection rates and to obtain material for laboratory study. The specimens obtained from surveys were kept in the laboratory and blood films were made at weekly intervals.

OBSERVATIONS

Observations were begun in July 1949 and continued through May 1950. Figure 1 shows dates of capture and results of blood film examinations of individual specimens; adults and juveniles are grouped separately. Table 1 gives the frequency distribution of captures of adult and juvenile lizards observed in this study. More than half of all the lizards were collected twice or more, and twelve of the seventeen lizards with positive blood smears were examined at least twice. Parasite densities, as measured by the number of parasites per 10,000 red blood cells, for each positive film are shown in table 2.

As indicated in figure 1, 12 of the 29 adult lizards and five of the 27 juveniles were positive for *P. floridense* at some time during the study. Positive blood films were

TABLE 1

Frequency distribution of number of captures of adult and juvenile Sceloporus u. undulatus

NUMBER OF TIMES CAPTURED	NUMBER OF LIZARDS	
	Adults	Juveniles
11	1	0
10	0	0
9	0	2
8	3	1
7	2	0
6	0	2
5	5	2
4	2	1
3	4	2
2	2	6
1	10	11
Total	29	27

collected from some of the adult lizards every month. During the first three months, 22 adult lizards were examined and nine were found positive. Until October there was no qualitative changes in parasitemias. As shown in figure 1, four positive and 11 negative specimens had been reexamined at the end of that month. After September, positive films were obtained from five specimens from which a series of negative films had been secured previously. These were P, AQ, AI, AA, and D. Three examinations of specimen P had been negative, but on October 24 a positive blood film was obtained. The parasitemia was very light; less than 10 parasites per 10,000 red blood cells were present. Two subsequent films from this individual, collected November 9 and January 30, also were found to have approximately the same density of parasites. Infections were found in AQ on January 23, AI on January 30, AA on April 28, and D on May 3. In the last instances negative films had been obtained a month or less before parasites were found. Two lizards, both adults, from which positive films were obtained were later found to be negative, specimens P and T.

No infections were observed in juvenile lizards until November. At the end of that

TABLE 2

Dates of capture and density of P. floridense *observed in blood films from* Sceloporus u. undulatus *collected in the intensive study area*

DATE OF CAPTURE	NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS																
	B	D	E	L	P	S	T	AA	AB	AI	AJ	AQ	AT	AV	AX	BA	BD
7-12-1949	68	−	53														
7-19-1949	111																
7-25-1949			55														
7-29-1949			52	325													
8-8-1949	320																
8-12-1949					−												
8-24-1949						135	15										
8-31-1949					−	1006											
9-13-1949					−												
9-16-1949	+																
9-28-1949								−	+								
10-13-1949		−						−									
10-18-1949							14										
10-21-1949								−									
10-24-1949					+												
10-28-1949						215											
11-4-1949									54	−							
11-10-1949		−			+						531						
11-21-1949	+							−	129	−							
11-28-1949	+										171	−					
12-8-1949	+									−							
12-17-1949	+												1594				
12-20-1949												−					
1-23-1950		−								−		+					
1-27-1950												29					
1-30-1950		−			+					19				3003			
2-8-1950															540		

2-13-1950												13							
2-27-1950												42							
3-17-1950	301										130	46						625	
3-24-1950									2317										
4-20-1950		−			−														
4-21-1950											707								
4-28-1950	675								60										241
5-3-1950	557	+			−														
5-10-1950								−										4074	

TABLE 3

Incidence of Plasmodium floridense *in* Sceloporus u. undulatus *collected in surveys*

YEAR AND MONTH	ADULTS		JUVENILES	
	No. captured	No. positive	No. captured	No. positive
1949				
June	6	2		
July	15	3		
August	9	1	11	
September	10	1	7	
October	12	4	17	
November	2		19	
December			1	
1950				
January	16	11	63	9
February				
March	2		1	
April	20	9	7	
May	29	3	27	1
June	7	2		
July	23	1	8	
August	13	3	6	
Total	164	40	167	10

TABLE 4

Monthly distribution of Sceloporus u. undulatus *positive for* Plasmodium floridense *indicating density and subsequent course of infection*

MONTH OF COLLECTION	NUMBER OF POSITIVE SPECIMENS	PARASITE DENSITY PER 10,000 RBC				SUBSEQUENT COURSE			
		<100	100–1000	1000–10,000	>10,000	Rising	Falling	Stable	No. data
1949									
June	2	1		1			2		
July	3	1	2			1	2		
August	1		1					1	
September	1		1			1			
October	4		2	2		2	1	1	
1950									
January	20	3	8	7	2	16	3		1
April	9	4	4	1		7	1	1	
May	4	2	2			1	3		
June	2								
July	1								
August	3								

month, 19 hatchlings of 1949 had been examined with only a single positive. Specimen AJ was found to be infected on November 10 and the film collected on November 8 also was positive. Subsequent to November, eight other juvenile lizards were examined and three were found to be parasitized on the initial examination. In one instance a positive film was obtained from a juvenile lizard which had been negative on previous examinations. Specimen AI was first observed October 28 and a negative blood film was obtained. Three other examinations of this specimen were made before a positive film was found on January 27. Subsequently, three additional positive films were obtained.

Table 3 shows by months the number of adult and juvenile lizards collected outside the intensive observation area. Lizards seek secluded places during the cold months and specimens cannot be obtained by ordinary collecting methods except on warm days when they leave the sheltered places. For this reason it was not possible to obtain comparable samples every month. Parasites were found in adult lizards each month that specimens were collected, with the exception of two months when only two individuals were captured. No infected juvenile lizards were found until January. Fifty-five lizards were examined during the last half of 1949 without a single infection being detected. Only one series of juvenile lizards, collected in January, was found to have an appreciable incidence of parasites.

Table 4 shows the level of parasitemia at the time of capture and the subsequent course of infections in positive lizards collected in surveys and followed in the laboratory for at least one month.

DISCUSSION

Although the number of animals involved in this study is not large, the data do provide some tenable indications as to the distribution of infections, period of transmission, and course of infections. In the following discussion some interpretations are absed upon various aspects of the development of *P. floridense*. However, not enough information is available from these or other studies to permit an accurate characterization of this infection. In general the data from Thompson's (1944) studies are followed. He observed in experimental blood-induced infections of *P. floridense* in *S. u. undulatus* that the prepatent period varied from 1 to 2 weeks, the period of acute rise required 60 to 70 days, and the patent period lasted about 3 months. Studies made on the course of natural and artificial infections in this laboratory are presented elsewhere (Goodwin and Stapleton, 1951).

Distribution of infections. Thompson and Huff (1944) called attention to the limited and intermittent geographical distribution of saurian malaria infections. In this study which was confined to a comparatively small area, about 300 square miles, there was no indication that foci of infection were restricted sharply. The area selected for intensive study had a comparatively high parasite rate. Although as detailed repetitive surveys were not conducted in other areas, results of examination of small series collected periodically from other restricted habitats indicated a wide geographic variation in the infection rate of *P. floridense*. The finding in the intensive area of 12 of 29 (41 per cent) adult lizards and five of 27 (18 per cent) juveniles positive during the course of the study provide an infection rate which was approximated in

only one other series of lizards examined. The collection of January (table 3) had 11 of 16 (69 per cent) adult and 9 of 63 (12 per cent) of the juvenile lizards that were positive for the infection. Although the monthly figures are small, incidence rates in the intensive area did not diverge greatly from the rate for the entire study period.

Period of transmission. One purpose of these observations was to obtain information on the seasonal variation of infections which might suggest a period of parasite transmission. With this knowledge, searches for possible vectors could be intensified just before and during the period of increasing natural incidence. Data from the intensive study area and from survey material suggest that transmission may be seasonal. Consider first the occurrence of parasites in animals from which negative films had been obtained previously. As indicated above, this was observed in five instances; specimens are designated D, P, AA, AI, and AQ (table 2). The first specimen observed, P, was found to carry parasites on October 24; three negative films had been collected previously. Two other positive films were obtained over a three month period and two additional negative films were later collected (figure 1). The parasite density in all of the positive films was less than 10 parasites per 10,000 red blood cells. The lack of sufficient observations prevented precise designation of the stage of this infection. Thompson (1944) has shown that inoculated infections may have a low peak intensity and a short patent period, presumably due to natural immunity. It is likely that such a response could take place in natural infections. The possibility of a relapse cannot be overlooked, although this phenomenon has not been observed with *P. floridense* in *S. u. undulatus*. (Thompson and Huff (1944) observed, however, the reappearance of parasites in two *Crotophytus collaris* infected with *P. mexicanum*.) If the infection in P was acquired just prior to the first positive film, transmission must have been effected in October, unless the prepatent period is unusually long.

Records from three specimens, which had patent infections when first observed, suggest the possibility of fall transmission. The infection in AJ was probably declining when first observed on November 10 since a later examination indicated a decrease from 531 to 171 parasites per 10,000 red blood cells. This juvenile lizard was probably infected in September or October. The infection in AB was below 10 parasites per 10,000 red blood cells when first observed on September 28. There was apparently a gradual rise in parasite density until the latter part of November. No further examinations were made until March 17 when the parasite level was about the same as when the specimen was examined in November. The film obtained at the last observation, on April 21, showed an increase to about 700 parasites per 10,000 red blood cells. It cannot be determined from these data whether the peak was reached between November and March and the rise in April represents a secondary increase, or if the acute rise covered the November to March period and the last count was made while the initial increase was developing. In either case, it seems likely that transmission was effected shortly before the last of September.

The other four cases which developed parasitemias following the collection of negative films all argue for a later transmission period. Low parasite levels were observed in specimens AI and AQ in January following four and two negative films,

respectively, obtained during the previous two months. The infection was observed for more than a month in AI without detection of a significant rise in the number of parasites. Thompson (1944) also observed a slow initial rise in some of his specimens. Only one film was obtained from AQ after the first positive one, and a slight increase in parasites was detected. Presumably, these cases could represent either new infections or relapses. In our opinion, the former assumption seems more tenable in view of the results obtained from observation of the series of negative lizards mentioned subsequently. In the case of AI, it is virtually certain that the infection was a new one. This lizard was a hatchling of 1949 and it is very unlikely that the infection could have been acquired, gone through a complete course in the lizard, and become negative in the time available.

Specimen AA was examined periodically over a period of seven months. Four negative films were obtained from September 28 through November 21. On the next examination, March 24, a positive film was obtained which showed a parasite count of about 2,300 parasites per 10,000 red blood cells. The film obtained at the last observation on April 28 had about 60 parasites per 10,000 red blood cells. This infection is believed to be a new transmission, the acute rise occurring between the last of November and the middle of March. The two observations on BA, a juvenile lizard, suggest that the infection in this specimen followed a similar course.

Six films obtained from D over a period of approximately ten months were negative for parasites. A positive film having less than 10 parasites per 10,000 red blood cells was obtained May 3. No subsequent examinations were made. It is probable that this was a primary infection, transmitted recently. It is not likely that a long series of negative films would be obtained in case of a prolonged prepatent period. In instances of a delayed initial rise, parasites are usually detectable but remain at a low level for an extended period, as demonstrated in the infection of AI. Thompson also observed this pattern of development in blood-induced infections.

Only one infection, that in specimen S, suggests the possibility of summer transmission. The infection in S was low when first observed on August 24. A week later the blood contained more than 1,000 parasites per 10,000 red blood cells. In approximately two months the parasitemia was down to around 200 parasites per 10,000 red blood cells.

Information on specimens B, E, and T is not indicative of a specific transmission period. The infections in E and T might simulate conditions in the development of a primary parasitemia but the small number of observations on these stable infections cannot be related to observations on other specimens from which a larger series of films was obtained. It is believed, however, that these infections are following the pattern of the one in B, exhibiting a low grade developed parasitemia.

Information obtained from lizard hatchlings also suggests that transmission occurred late in the year. At the end of November, 19 juvenile lizards had been examined and only one, AJ, was found positive; the course of this infection was described above. Other juvenile lizards found positive were AI and AV on January 29, AX on February 7, and BA on March 17. The height of parasitemia observed and the development of infections in the specimens from which more than one film was obtained, also suggest that transmission occurred in the fall or winter.

The possibility of transmission during this season is further attested by data from the lizards collected in surveys outside of the intensive study area. The data in table 3 may be affected by variations in infection rates encountered in different locations, although no effort was made to select areas for survey on the basis of probable parasite rates. The August, November, and December collections were from the same location and seasonal rates can be compared without this reservation. Two inferences from these data are believed to be significant: the greatest proportion of lizards were found infected in the fall and winter months, and no juvenile lizards were found with parasites until January. Data on positive specimens given in table 4 also suggests that natural infections were higher in winter and the proportion of parasitemias that subsequently showed an increase was greater in winter than at other seasons.

Inference of seasonal characteristics of occurrence of *P. floridense* from these data must necessarily be made with reservations. In all probability, climatic conditions influence appreciably the abundance of vectors and activity of vector and vertebrate host. The winters of 1948-49 and 1949-50 were unusually mild; January 1950 was especially warm. This may have been the causal factor responsible for the conditions observed. Generalizations cannot be made accurately unless studies are made over a longer period in a variety of climatological situations.

Course of infection. In most instances the infections followed in nature conform to the courses of induced infections observed by Thompson (1944). Two infections observed in this study suggest that the patent period may be significantly longer than approximately three months as observed by Thompson. Parasites were demonstrable in each of the 11 films collected from specimen B from July 12 through May 3. The variation in parasite density recorded for this individual is believed to be within the range of fluctuation of a stable infection, although a patent period of this length has not been observed in these or other studies. The other infection, in AB, was observed 6 times between September 28 and April 21. As indicated previously, available data do not indicate whether this parasitemia was increasing over this period or if a peak was reached and decline began between examinations. At any rate, the patent period in this instance lasted for about 7 months. The possibility of reinfection cannot be overlooked in these cases as work on superinfections has not been reported. The gradual increase in parasites observed in AI over a period of 6 weeks also was noted by Thompson in artificial infections.

In attempts to obtain information on the approximate prepatent period and relapse rate of natural *P. floridense* infections, specimens collected in surveys and found parasite-negative on first examination were held in the laboratory where blood films were obtained and examined weekly. If infections developed, they became patent within two weeks. No infections were detected after the specimens had been under observation for 14 days although 66 specimens were observed for 1 month, 18 for 2 months, 7 for 3, 9 for 4, 5 for 5, and 2 for 6 months. These data suggest that the incubation period could not be much longer than two weeks.

SUMMARY

In connection with investigations of infectivity of *Anopheles* mosquitoes to sylvan malarias, studies were made of the incidence of *Plasmodium floridense* in *Sceloporus*

u. undulatus over a ten month period. Repeated examinations were made of individual specimens comprising a population in a two acre area and surveys were made of lizards collected from other areas. The following observations were made:

1. Infections were observed among adult lizards during each month of the study but no parasites were found in hatchlings of the current year until November.

2. More lizards were found infected during the fall and winter months and a greater proportion of these infections subsequently increased than those in specimens collected at other times of the year.

3. Observation of specimens maintained in the laboratory suggested that the prepatent period following natural infection is probably about two weeks.

4. The patent period was observed for at least seven months in one individual and for ten months in another.

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RESUMEN

Con relación a investigaciones sobre la infectividad de mosquitos *Anófeles* con malarias silvestres, se llevaron a cabo estudios acerca de la incidencia de *Plasmodium floridense* en *Sceloporus u. undulatus* por un período de diez meses. Exámenes repetidos de especímenes individuales se hicieron abarcando una población de dos acres y se observaron lagartos procedentes de otras áreas. Se hicieron las siguientes observaciones.

1. Se observaron infecciones en lagartos adultos durante cada mes del estudio pero no se encontraron parásitos en los recién nacidos del corriente año hasta noviembre.

2. Se encontraron más lagartos infectados durante los meses de otoño e invierno y estas infecciones aumentaron subsiguientemente en proporción mayor que las de los especímenes recogidos durante las otras estaciones del año.

3. La observación de ejemplares mantenidos en el laboratorio sugirió que el período de incubación siguiente a la infección natural es probablemente cerca de dos semanas.

4. El período de infección patente se observó por lo menos por siete meses en un individuo y por diez meses en otro.

STRAIN DIFFERENCES IN *PLASMODIUM GALLINACEUM* BRUMPT

II. EXPERIENCES WITH THE SPOROZOITE AND SINGLE OOCYST PASSAGE OF THE BI STAIN

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In a previous report (Greenberg *et al.*, 1950) it was shown that two different strains, designated the BI and the SP, were derived from the 8A strain of *Plasmodium gallinaceum*. Both these strains produce abundant phanerozoites (late tissue parasites), and both show identical infection patterns when transferred by the inoculation of infected blood. The phanerozoites of the SP strain, however, are able to produce merozoites capable of developing normally in the erythrocytes while those of the BI strain cannot. Developed infections produced by sporozoites of the SP strain (Coatney *et al.*, 1945b) follow the description of Huff and Coulston (1944) in that phanerozoites, gametocytes, and trophozoites occur concurrently. On the other hand, sporozoites of the BI strain produce infections which are almost exclusively exo-erythrocytic (Haas *et al.*, 1946 and 1948).

Haas *et al.* (1948) reported that the sporozoites of the BI strain occasionally produced infections showing gametocytes. By selecting such infections for mosquito passage these investigators were able to isolate a strain which produced trophozoites and gametocytes but phanerozoites only rarely. This paper describes our failure to duplicate this strain conversion by the usual methods of injecting infected glands or pooled sporozoite suspensions. However, by the inoculation of single oocysts we were able to obtain infections no longer characteristic of the BI strain but indistinguishable from those produced by the SP strain.

MATERIALS AND METHODS

New Hampshire Red chicks, one week old and weighing 45 to 65 grams, served as vertebrate hosts and insectary-reared *Aedes aegypti* mosquitoes served as vectors. Sporozoites were injected in dissected salivary glands, in pooled suspensions, or in intact oocysts. The infected glands and the sporozoite suspensions were prepared and injected according to the methods of Coatney *et al.* (1945a and b).

Single intact oocysts were obtained for inoculation by (1) the selection of whole midguts containing only one oocyst, or (2) by dissecting a portion of a midgut with only one oocyst attached. The inoculation of midguts, whole or in part, followed the same procedure as that used for salivary gland injections.

Smears of the peripheral blood of all chicks were begun on a day which would permit the demonstration of one day's negative parasitemia before patency. Smear preparations of the brain cortex were made from most chicks on the day of death or when killed. Methods for the preparation of blood and brain smears, for the enumer-

ation of blood parasites, and for the estimation of exoerythrocyte densities are described in the first paper of this series (Greenberg et al., 1950).

EXPERIMENTAL

Two groups of chicks, 20 and 100 birds respectively, were inoculated with 1.0 mosquito equivalent of sporozoites from mosquitoes which had previously fed on chicks carrying the blood-passaged BI strain. One hundred nineteen birds became infected and all died with overwhelming phanerozoite (EE) infections (table 1). The mean day of death occurred 1.5 days after the first day of parasitemia. While sixty per cent of the chicks exhibited parasitized erythrocytes, none of the parasites was pigmented; less than 10 per cent of the chicks exhibited a peak parasite count of more than 10 parasitized erythrocytes per 10^4 red blood cells.

TABLE 1

Comparison of Plasmodium gallinaceum *infections in chicks injected with decreasing concentrations of sporozoite inocula (BI strain)*

MOSQUITO EQUIVALENTS	NO. INFECTED / NO. INJECTED	PER CENT WITH PARASITEMIA	MEAN PREPATENT PERIOD[1]	NO. OF CHICKS WITH PEAK PARASITE COUNTS OF										MEAN DAY OF DEATH
				0	5	10	20	30	40	50	80	110	1600	
			days	*parasitized erythrocytes/10^4 erythrocytes*										
5.0	20/20	90.0	7.8	2	7	10	0	0	1	0	0	0	0	9.2
1.0	119/120	60.3	9.4	44[2]	36	22	5	3	0	1	0	0	0	10.9
0.1	30/30	60.0	10.3	12	7	9	0	1	0	0	0	1	0	11.4
.01	86/90	56.9	11.2	37	18	17	10	1	1	0	1	0	1[3]	12.6
.001	16/30	56.2	13.8	7	4	5	0	0	0	0	0	0	0	15.2
Total	271/290	—	—	102	72	63	15	5	2	1	1	1	1	—

[1] Only chicks with parasitemia are included.

[2] Peak levels at 1.0 mosquito equivalents are complete for 112 chicks.

[3] Chronic. All other infected chicks died.

In attempts to modify the infection so as to obtain a normal parasitemia, four groups of chicks, 170 birds in all, were inoculated with sporozoite dosages of 5.0, 0.1, 0.01, and 0.001 mosquito equivalent. All the chicks inoculated with 5.0 mosquito equivalents became infected; 90 per cent exhibited unpigmented erythrocytic parasites but only one chick showed a peak count of more than 10 parasitized erythrocytes per 10^4 red blood cells (table 1). The mean period of survival after the appearance of parasites in the blood was 1.4 days, and death occurred in all instances with 3+ and 4+ brain infections. With decreasing concentrations of the sporozoite inoculum there was a gradual decrease in the infection rate, number of chicks exhibiting parasitemia, and a delay in the mean day of death (table 1).

Of the 290 chicks used in the experiments described above, 271 became infected, 270 showed EE infections and only one (Chick A, figure 1), the 283rd chick inoculated, developed an infection with pigmented, segmenting erythrocytic parasites. The infection in this bird became patent on day 17 after inoculation and reached a peak count of 1,600 parasitized erythrocytes per 10^4 red blood cells on day 24. When

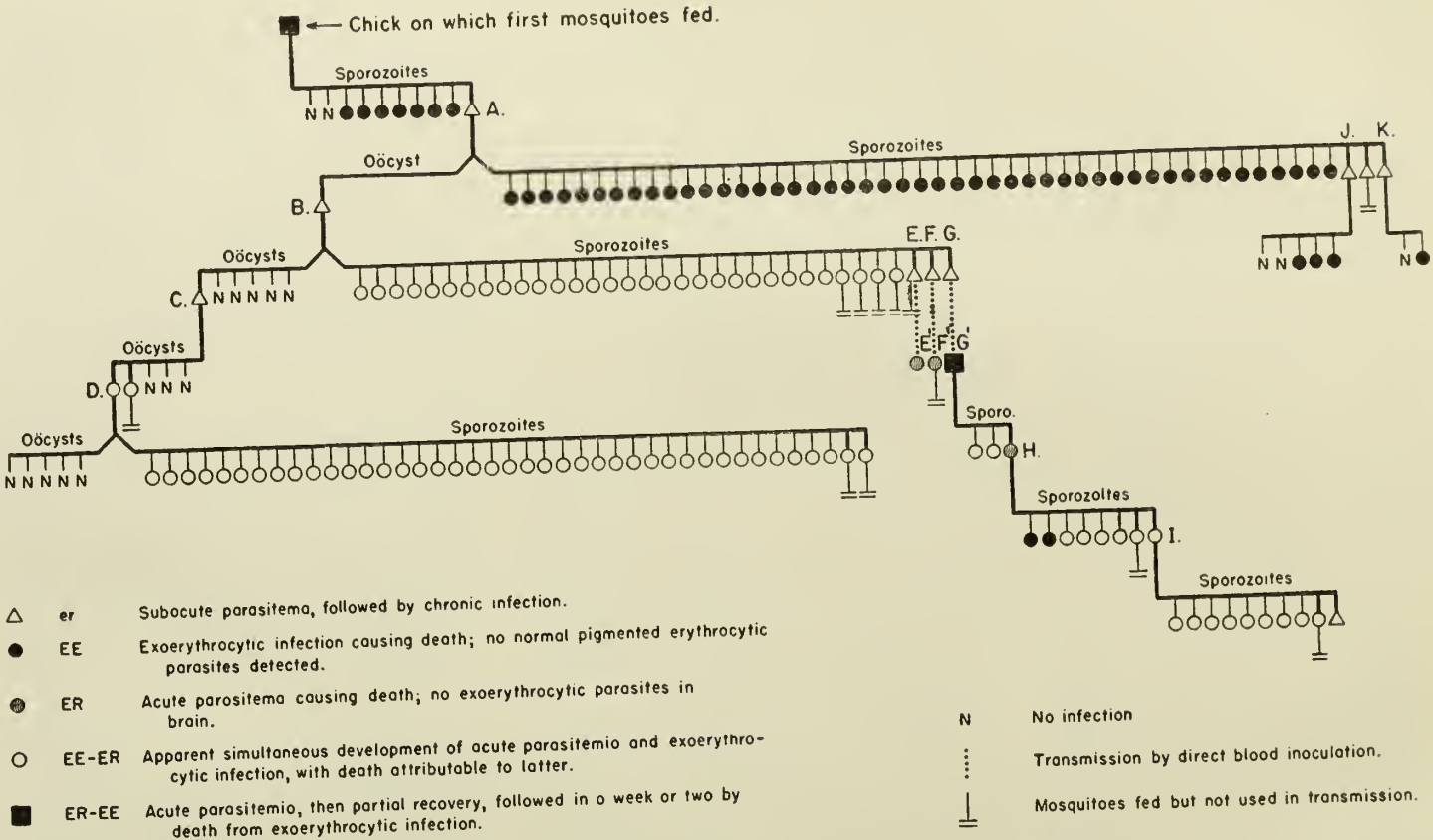

FIG. 1. Results of the mosquito-passage of the BI strain of *Plasmodium gallinaceum*.

the parasite count was 90 parasitized cells per 10^4 erythrocytes, mosquitoes were allowed to feed and they became infected. The chick was killed 35 days after inoculation, but no brain smear was made. The infection was subsequently serially passaged by the inoculation of (a) sporozoites or (b) single oocysts (figure 1).

Sporozoites from mosquitoes fed on Chick A produced predominantly EE infections (death due to exoerythrocytic parasites, no normal erythrocytic parasites); only three of 50 chicks infected exhibited normal erythrocytic parasites. This was an increase in the relative number of chicks having normal erythrocytic parasites as compared with the original BI strain (table 1). However, when the strain was again

TABLE 2

Infections resulting from injections of single oocysts

DONOR	NUMBER OF DAYS AFTER INFECTIOUS FEEDING	MIDGUT	OOCYST SIZE	RESULTING INFECTION
A[1]	9	entire	medium	er
B	9	entire	large	negative
	9	entire	small	er
	9	entire	very large	negative
	10	part	large	negative
	10	entire	small	negative
	10	entire	small	negative
C	9	part	medium	negative
	9	entire	medium	negative
	9	part	medium	negative
	9	entire	large	EE-ER
	9	entire	large	EE-ER
D	12	entire	large	negative
	12	entire	small	negative
	15	entire	small	negative
	15	part	large	negative
	15	entire	large	negative

[1] Letters refer to Figure 1.

passaged (from J and K) by sporozoites (in infected salivary glands), three of the seven chicks inoculated failed to become infected; four developed EE infections and died. Therefore, this line of passage could not be continued.

The infection from Chick A was serially passaged three consecutive times (B, C and D) by the injection of single oocysts. In all, four of 12 chicks developed infections (table 2); two exhibited er infections (subacute parasitemia, infections becoming chronic) and the other two, EE-ER infections (death due to exoerythrocytic parasites, acute parasitemia present). Five chicks were inoculated with single oocysts for the fourth oocyst passage; none became infected and the line was therefore lost.

Sporozoites from the infection in Chick B (figure 1) produced predominantly EE-ER infections in contrast to those from Chick A (figure 1) which produced predom-

inantly EE infections. After one oocyst transfer, the strain had changed its characteristics and was not further modified through oocyst transfers at C and D as shown by comparison of sporozoite-induced infections at B and D. In addition, the strain showed no further recognizable changes through one blood transfer (recipients E′, F′ and G′) and serial sporozoite transfers from G′, H and I.

A final experiment was carried out in an attempt to answer the question whether single oocysts derived from an unselected donor carrying the blood-passaged BI strain (table 2) would result in infections showing erythrocytic forms. This was found not to be true, for out of 10 chicks inoculated, only one became infected; it died of an overwhelming EE infection.

DISCUSSION

The blood-inoculated BI strain of *Plasmodium gallinaceum* was used as the starting point from which we attempted to develop separate strains by means of mosquito passage with special emphasis on initiating the infections by single oocyst inoculations.

In an earlier study, Coatney *et al.* (1945b) reported on the course of infection in 439 chicks inoculated with 1.0 mosquito equivalent of sporozoites of the SP strain. All of these birds had typical EE-ER infections, exhibited normally parasitized erythrocytes, 87 per cent had peak counts of 1,000 or more parasitized erythrocytes per 10^4 red blood cells, and a mean survival after initial patency of 3.4 days. This was in marked contrast to our results with the BI strain. Of the 119 chicks infected, using 1.0 mosquito equivalent of sporozoites, all had typical EE infection patterns, 60 per cent showed nonpigmented erythrocytic parasites, none had peak counts of over 1,000 parasitized erythrocytes per 10^4 red blood cells, and the average survival after initial patency was only 1.4 days. This is in essential agreement with the findings of Haas *et al.* (1948) with this same strain.

Mosquitoes which had fed on typical EE patterns of infection failed to become infected and consequently efforts were made toward modification. It was thought (1) that an increased dosage of sporozoites might produce an infection in which a normal parasitemia would occur before the maturation of phanerozoites could bring about death of the birds, or (2) that a much smaller inoculum might allow a gradual increase in asexual blood forms, with concomitant gametocytes, to an appreciable level before death would occur from exoerythrocytic parasites. However, when the number of sporozoites in the inoculum was varied, it served to modify only the infection rate, the number of chicks with aberrant blood parasites, the mean prepatent period, and the mean day of death but not the peak parasite counts and the mean survival period after patency.

The spontaneous appearance of a single chronic infection (Chick A, figure 1) among 271 birds, is, at this time, an inexplicable phenomenon but its fortuitous occurrence nevertheless afforded an opportunity for further sporozoite passage of the strain. Haas *et al.* (1948) and Haas (personal communication) had found that such infections occurred in the sporozoite-transmitted BI strain in approximately the same ratio as ours; after successive passages, using pooled sporozoites, the proportion of chicks

demonstrating normal parasitemia increased while those exhibiting phanerozoites decreased until finally infections were produced in which practically all of the infected chicks had normal parasitemia and only rarely were phanerozoites produced.

In our work, after one sporozoite passage from Chick A, the proportion of chicks with normal parasitemia increased from one in 271 to three in 50. However, at the second passage, due to the paucity of infected mosquitoes, only seven chicks could be injected. Four of these developed EE infections and the strain was lost. One may speculate that had it been possible to inoculate a large number of chicks some might have developed chronic infections and the line could have been continued as Haas and his coworkers had been able to do.

Concurrently, we employed another approach to the problem of strain conversion. Assuming the ability of the parasite to produce a normal parasitemia to be genetically controlled it seemed logical to attempt to derive an infection from the product of one syngamy and to this end single oocyst inoculations were attempted.

Only one oocyst could be isolated for the first passage. The resulting infection (Chick B, Figure1) was indeed a fortunate occurrence as can be seen by examination of the chart (figure 1). This is, to our knowledge, the first report of an infection resulting from the injection of a single intact oocyst, although Missiroli (1937) and Neri (1937) record infections resulting from the inoculation of emulsions of crushed oocysts and haemolymph containing sporozoites, while Shute (1943) obtained infections by injecting sporozoites from crushed oocysts.

After this passage, by a single oocyst, the transformation in strain characteristics was spectacular in that all sporozoite infections derived from Chick B (figure 1) exhibited normal parasitemia. Furthermore, the strain was stable in its ability to continue to produce such infections even when pooled sporozoites were used. It should be noted, however, that the strain produced abundant phanerozoites; and in this and other respects it is indistinguishable from the SP strain as described by Coatney *et al.* (1945b) and does not compare with the phanerozoiteless strain derived by Haas *et al.* (1948), or the original BI strain.

It is not unlikely that the appearance of a chick with a chronic, er, infection is necessary before strain conversion can be effected. When single oocysts rather than pooled sporozoites from mosquitoes which had fed on an unselected chick carrying the blood-passaged BI strain were inoculated into 10 chicks, the one resulting infection was an EE pattern, indistinguishable from that of the typical sporozoite-passaged BI strain.

The evidence presented in this report strongly suggests that the ability to produce normal parasitemia is genetically controlled. However, it is difficult on this basis to explain the spontaneous appearance of the chronic infection in Chick A (figure 1).

SUMMARY

1. Two hundred seventy-one out of 290 chicks inoculated with varying numbers of sporozoites of the blood-passaged BI strain of *Plasmodium galinaceum*, became infected. The pattern of infection produced was not affected by the numbers of sporozoites inoculated. Two hundred seventy of the chicks died with overwhelming

exoerythrocytic infections, with no normal pigmented erythrocytic parasites detected (EE pattern); and only one chick developed a normal parasitemia and later became chronic (er pattern).

2. Sporozoite passage from this chronic infection resulted in a predominance of EE infections through two transfers.

3. A single oocyst inoculation derived from this chronic infection was successful, and a chronic infection resulted. This infection was further passaged twice by single oocyst inoculation, but the third passage was unsuccessful.

4. As the result of oocyst transfer the strain was modified i.e. normal parasitemia with gametocytes was produced.

5. Inoculation of oocysts from blood-passaged BI strain infections produced only an EE infection.

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RESUMEN

1. De 290 pollos inoculados con una cantidad variable de esporozoítos pertenecientes a la cepa BI de *Plasmodium gallinaceum* mantenida a través de pases de sangre, 271 fueron infectados. El tipo de infección producido no fué afectado por el número de esporozoítos inoculados. 270 de los pollos infectados resultaron muertos con infecciones exoeritrocíticas irresistibles pero no se hallaron parásitos eritrocíticos normalmente pigmentados (Tipo EE); solamente uno de los pollos desarrolló una parasitemia normal el cual más tarde se volvió crónico.

2. El pase de esporozoítos pertenecientes a esta infección crónica resultó en una predominancia de infecciones del tipo EE después de dos pases.

3. La inoculación de un solo oocito derivado de esta infección crónica fué efectiva, produciendo una infección crónica. Esta infección fué nuevamente transferida dos veces por medio de inoculacienes de un solo oocito pero un tercer pase no resultó efectivo.

4. Como resultado del pase mediante oocitos la cepa se alteró, esto es, se produjo parasitemia normal con gametocitos.

5. La inoculación de oocitos procedentes de infecciones con la cepa BI mediante transferencias de sangre produjo únicamente la infección del tipo EE.

STRAIN DIFFERENCES IN *PLASMODIUM GALLINACEUM* BRUMPT

III. THE SPONTANEOUS CONVERSION OF A PHANEROZOITE-PRODUCING SP STRAIN TO A PHANEROZOITELESS M STRAIN THROUGH MOSQUITO PASSAGE

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The sporozoites of the SP strain of *Plasmodium gallinaceum* characteristically produce infections in which phanerozoites (late exoerythrocytic parasites), trophozoites and gametocytes develop concurrently (Coatney *et al.*, 1945, 1945a, 1945b and Greenberg *et al.*, 1950). Greenberg *et al.*, (1950a) showed that by using 0.01 mosquito equivalent of sporozoites of this strain to initiate an infection the curative activity of certain 8-aminoquinolines could be demonstrated. As the curative-testing of these compounds progressed it was discovered that the length of the prepatent period was increasing and that the infectivity and mortality rates were decreasing with the result that data on several test groups had to be discarded. It then became necessary to learn if the strain had been modified in some way or whether our techniques were faulty. To check on the immutability of the strain we ran test series employing the one-mosquito equivalent technique of Coatney *et al.*, (1945b) and discovered that the strain had lost its ability to produce phanerozoites and in this respect resembled a strain described by Haas *et al.*, (1948) which we have designated the M strain.

The passage history of the SP strain will be dealt with in this report and the circumstances described which operated to effect the alteration in its behavior.

METHODS

Figure 1 presents the passage history of the SP strain of *P. gallinaceum* from August 1947 to November 1949. In the routine strain passages young New Hampshire Red chicks were inoculated in the pectoral region with 0.01 to 1.0 mosquito equivalents of sporozoites according to the method of Coatney *et al.*, (1945b). The purpose of these inoculations was to maintain the strain; consequently, no data are available on the type of infections produced in the recipient chicks. After donors were selected for further passage of the strain, on or about the tenth day after inoculation, the surviving chicks were destroyed and no autopsies were performed. In all these strain passages the vectors were laboratory-reared *Aedes aegypti* mosquitoes.

Periodically, large groups of week-old chicks weighing 45 to 65 grams were inoculated with sporozoites for the purpose of testing the antimalarial activity of chemical compounds. All the test series except C′ were inoculated with 0.01 mosquito equivalent according to the method of Greenberg *et al.*, (1950). The chicks in series C′ were inoculated with 1.0 mosquito equivalents. Series K through P in which the inoculum was 1.0 mosquito equivalent, were not used for drug tests. Those series in

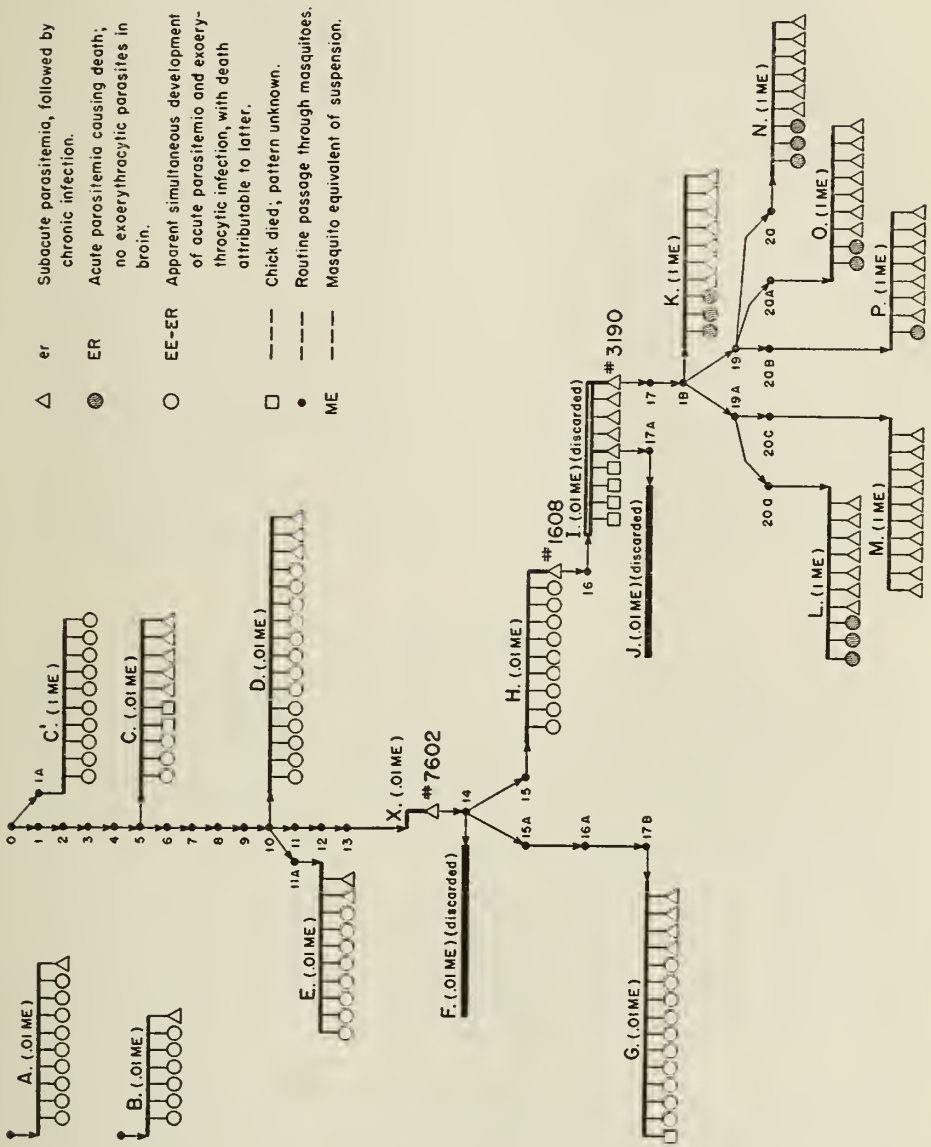

FIG. 1. Pattern changes in mosquito-transmitted *Plasmodium gallinaceum*.

which the inoculum was 0.01 mosquito equivalents were discarded if less than 50 per cent of the chicks had patent infections by the tenth day after inoculation. In such discarded series the chicks were killed on the 10th or 11th day and no autopsies were performed. Therefore, data on the types of infections produced are not available, except for chick No. 7602 and five chicks of series I, which were allowed to live after they had served as donors for strain passage. For those series which were used for drug evaluation the data presented in figure 1 were from untreated controls.

In all complete series, the presence of typical erythrocytic parasites, stained with Giemsa, was ascertained at least once for each chick. Furthermore, a brain-smear was made of all but two chicks (series C) dying during a 30-day observation period, or killed thereafter and examined (Coatney *et al.*, 1945b) for the presence and density of phanerozoites. In series L through P detailed observations on daily parasitemia were made for each chick for the first three weeks after inoculation.

Series A and B are shown as examples of the patterns of infections produced in untreated control chicks inoculated with 0.01 mosquito equivalents of sporozoites of the SP strain derived from lines of passage parallel to the numbered transfers shown in figure 1.

The types of infections encountered in these studies have been designated as in the two previous papers of this series (Greenberg *et al.*, 1950a, and Trembley *et al.*, 1951).

EXPERIMENTAL

The passage history of the material discussed here begins with series C′ in which each chick was inoculated with 1.0 mosquito equivalent of the SP strain. All the chicks died of EE-ER infections; phanerozoites were abundant and were considered to be the chief cause of death. In series C five chicks died; three exhibited phanerozoites and on two, no brain smears were made. Series D and E were normal for the strain, i.e., most of the chicks died of phanerozoite infections which developed concurrently with erythrocytic parasites.

Series X was discarded because the infectivity rate was too low, but one chick, No. 7602, which first had detectable parasitemia on day 11, was used on the following day to infect mosquitoes for further passage of the strain. This chick survived the infection and was later destroyed. Subsequently, series F also had to be discarded but series G and H were typical in that most of the chicks died of phanerozoite infections.

Chick No. 1608 in series H served as a donor to infect mosquitoes for continuing the strain; this bird later developed a chronic infection. Series I was aberrant in that the infectivity rate and mortality rate were low, and the series was discarded. However, two of the chicks, which eventually survived the infection, were used as donors for different lots of mosquitoes. One of these lots was used to infect the chicks of series J, which was later discarded. The other lot of mosquitoes was used to continue the strain. Two routine passages after series J, a group of chicks was inoculated with 1.0 mosquito equivalent of sporozoites (series K). None of the chicks was found to have phanerozoites; three died as a result of the parasitemia and the remainder survived until destroyed. When series C is compared with series K a marked difference

in the behavior of the strain is revealed after 14 passages, covering a period of approximately 14 months.

It was clear at this point that the strain was behaving atypically. In addition to the lot of mosquitoes used to infect series K there were two additional lots available originating from No. 3190. Each of these lots was used to infect a group of chicks, which were further used to divide the strain so that there were eventually five lots of mosquitoes carrying sporozoites derived from the infection in chick No. 3190 (20, 20A, 20B, 20C, 20D). Each of these lots of mosquitoes was used to infect ten chicks; 1.0 mosquito equivalent of sporozoites was given to each chick. At the same time (data not included in figure 1) ten chicks were inoculated with 1.0 mosquito equivalent of sporozoites of the M strain, which previously had been obtained from Dr. Haas. Forty-six of the chicks in series L, M, N, O and P became infected but none died of causes attributable to phanerozoites, although one chick in each of series N and O, after an extensive search, was found to have one dubious tissue form in the brain smears made at autopsy. It is noteworthy that one of the chicks in the series inoculated with sporozoites of the M strain (Haas *et al.*) was found to have one questionable phanerozoite in a brain smear made at autopsy. Two of the chicks in this group died of causes other than malaria, two died of parasitemia and six survived the infection until killed.

DISCUSSION

Prior to the passage history described in this paper the SP strain of *P. gallinaceum* had been transmitted by consecutive mosquito-chick passage for over seven years without essential change in the type of infections produced. Furthermore, another line was carried by blood passage simultaneously with the present one and subsequently passaged through mosquitoes with no loss of virulence or ability to produce phanerozoites. Finally, 0.01 mosquito equivalents of sporozoites were used for over a year (series A through G) to infect chicks for testing antimalarial drugs and during this time the infections were predictable; the infectivity rate by day 10 was over 50 per cent and the mortality rate in untreated chicks was over 75 per cent.

The change from a parasite which produced virulent infections with phanerozoites to one which was relatively non-virulent and produced few phanerozoites is striking. In its final form the strain was almost identical with the phanerozoiteless strain described by Haas *et al.*, (1948) which they obtained from a virulent BI strain of the parasite by similar methods.

In seeking to explain the change which occurred in a hitherto stable strain, the one fact which is apparent is that in three almost successive strain passages chicks with chronic infections happened to be chosen as donors (Nos. 7602, 1608 and 3190). Under normal passage conditions such a series of events would be most improbable because chicks receiving 0.25 or more mosquito equivalents of sporozoites seldom survive the infection. In the present passage history several of the series of chicks were inoculated with 0.01 mosquito equivalent of sporozoites, and as has been shown by Greenberg *et al.*, (1950), the probability of chicks developing chronic infections increases with the decrease in inocula to 0.01 or below.

It would appear that in order to produce a change in the strain as described here,

it is necessary to select chicks as donors which have survived infections resulting from small inocula of sporozoites.

While the mechanics of producing an alteration in strain characteristics are relatively clear, the fundamental changes which occur are obscure. It is difficult to know whether the change is a manifestation of an alteration in gene structure or is the result of changes in the parasite more or less divorced from genetic control.

SUMMARY

The history of the cyclical passage of the SP strain of *Plasmodium gallinaceum* has been described covering a period of approximately 27 months and through 20 consecutive mosquito transfers. In the early passages the strain was characterized by a high infectivity rate, such that practically all of the chicks inoculated with 0.01 mosquito equivalent of sporozoites became infected. The mortality rate was equally high, most of the chicks dying of causes attributable to phanerozoites. Between the 13th and 15th passages a change in strain characteristics occurred. The infectivity rate declined, as judged by the numbers of chicks infected on the 11th day following the inoculation of 0.01 mosquito equivalent of sporozoites. After the 15th passage none of the chicks was found to have died of causes attributable to exoerythrocytic parasites, even when 1.0 mosquito equivalent of sporozoites was inoculated. In examining the history of the strain passage it was found that between the 13th and the 15th passages several nearly consecutive donors had been used which had survived the infection. This fact is presented as a possible explanation of the change in characteristics of the strain.

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RESUMEN

Se ha descrito la historia del pasaje cíclico de la cepa SP de *Plasmodium gallinaceum* abarcando un período de 27 meses aproximadamente a través de 20 transferencias

consecutivas en mosquitos. En los primeros pases la cepa se caracterizó por un alto grado de infectividad de manera que casi todos los polluelos inoculados con 0.01 de un mosquito-equivalente de esporozoítos resultaron infectados. La proporción de mortalidad fué igualmente alta y la mayor parte de los pollos murieron de causas atribuíbles a fanerozoítos. Entre los pases trece y quince sucedió un cambio en las características de la cepa. El grado de infectividad declinó, juzgando por el número de pollos infectados en el undécimo día siguiente a la inoculación de 0.01 de un mosquito-equivalente de esporozoítos. Después del pase decimoquinto ningún pollo murió de causas atribuíbles a parásitos exoeritrocíticos, incluso cuando se inoculó 1.0 mosquito-equivalente de esporozoítos. Al revisar la historia de los pases de esta cepa se descubrió que entre los pases trece y quince se habían empleado donadores casi consecutivos que sobrevivieron la infección. Este hecho se presenta como una posible explicación del cambio de características en la cepa.

STRAIN DIFFERENCES IN *PLASMODIUM GALLINACEUM* BRUMPT

IV. EXPERIENCES WITH THE BLOOD PASSAGE OF THE PHANEROZOITELESS M STRAIN

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Numerous investigators have shown that phanerozoites, late tissue forms, are extremely abundant in the capillaries of the brain and other organs of most sporozoite-induced *Plasmodium gallinaceum* infections in the chick (James and Tate, 1938; James, 1939; Mudrow, 1940; Vitello, 1940; Huff and Coulston, 1944; Coulston *et al.*, 1945; Coatney *et al.*, 1945, 1945a, 1945b). On the other hand, Haas *et al.* (1948) have described a strain of this parasite in which sporozoite-induced infections produced few or no phanerozoites. According to these investigators this strain maintained its phanerozoiteless characteristics through several years of consecutive mosquito-chick passages. Dr. Haas kindly furnished us with chicks infected with this strain which we have maintained for 98 weeks, through 22 consecutive mosquito passages. Four hundred and twenty chicks have been infected with sporozoites of this strain, which we have designated the M strain, and in only one instance have phanerozoites been found in compression smears of the brains of these chicks, whether the birds died of the infection or survived it (Trembley *et al.*, 1951).

It has been found that phanerozoites will cause the death of most chicks which have survived the initial peak parasitemia induced by the intravenous inoculation of infected blood (James, 1939; Tullis, 1947; Haas *et al.*, 1948; Lewert, 1948). The blood parasites of two strains of *P. gallinaceum* (SP and BI) which we have investigated (Greenberg *et al.*, 1950) both characteristically produce infections exhibiting abundant phanerozoites. These phanerozoites are not detectable until the 12th day after the inoculation of infected blood (Tullis, 1947) and do not cause death until 16 to 24 days after inoculation (Haas *et al.*, 1948).

The present report deals with the behavior of the M strain of *P. gallinaceum* when subjected to consecutive blood transfer.

MATERIALS AND METHODS

The strain of *P. gallinaceum*, which we have designated the M strain, characterized by a paucity of phanerozoites in sporozoite-induced infections (Haas *et al.*, 1948) was used throughout this study. The hosts were week-old, New Hampshire Red chicks, weighing 45 to 55 grams. The method of inoculating the chicks with infected blood as well as the method for estimating parasitemia have been described in detail by Coatney and Sebrell, 1946.

Each of the four series described in this paper was begun by inoculating chicks with infected erythrocytes from a donor which had received its infection by the

inoculation of sporozoites. Subsequent transfers were made into five to 20 chicks using the infected blood of a donor chosen at random. The blood of the donor was

TABLE 1

Series A. Blood-passage of the M strain of Plasmodium gallinaceum

BLOOD PASSAGE NUMBER	NO. CHICKS SURVIVED* / NO. CHICKS INFECTED	NO. CHICKS DIED WITH NO PHANEROZOITES	NO. CHICKS DIED WITH PHANEROZOITES
1	1/20	19	0
2	11/15	4	0
3	0/10	9	1†
4	6/10	4	0
5	10/10	0	0
6	5/10	5	0
7	3/5	2	0
8	6/10	4	0
9	3/10	7	0
10	7/10	3	0
11	4/10	6	0
12	7/10	3	0
13	6/10	4	0
14	10/10	0	0
15	10/10	0	0
16	0/10	10	0
17	3/5	2	0
18	4/5	1	0
19	5/5	0	0
20	4/5	1	0
21	5/5	0	0
22	1/5	4	0
23	1/5	1	3‡
24	1/5	4	0
25	0/10	9	1
26	0/5	3	2
27	0/6	2	4
28	0/5	4	1
29	0/5	3	2
30	1/5	4	0
31	0/5	4	1
32	0/5	4	1
33	0/5	4	1
34	0/5	5	0
35	0/5	2	3
36	0/5	5	0
37	0/5	5	0

* Thirty days after inoculation

† Not a donor for next passage

‡ One of these chicks was donor for next passage

diluted with 0.85 per cent NaCl so that each chick received from 10,000 to 100,000 parasitized erythrocytes in 0.1 ml. of inoculum. On the 7th day after the inoculation, blood smears were made from all the chicks. The slides were stained with Giemsa and

TABLE 2

Series B. Blood-passage of the M strain of Plasmodium gallinaceum

BLOOD PASSAGE NUMBER	NO. CHICKS SURVIVED* / NO. CHICKS INFECTED	NO. CHICKS DIED WITH NO PHANEROZOITES	NO. CHICKS DIED WITH PHANEROZOITES
1	1/5	3	1†
2	0/5	5	0
3	1/5	4	0
4	2/5	3	0
5	4/5	1	0
6	0/5	5	0
7	3/5	2	0
8	3/5	2	0
9	4/5	1	0
10	3/5	1	1†
11	2/5	2	1†
12	1/5	4	0
13	2/5	3	0
14	3/5	2	0
15	3/5	2	0
16	4/5	1	0
17	4/5	1	0
18	4/5	1	0
19	4/5	1	0
20	3/5	2	0
21	5/5	0	0
22	2/5	2	1‡
23	5/5	0	0
24	5/5	0	0
25	5/5	0	0
26	5/5	0	0
27	3/5	2	0
28	5/5	0	0
29	5/5	0	0
30	4/5	1	0
31	4/5	1	0
32	4/5	1	0
33	2/5	3	0
34	3/5	2	0
35	2/5	1	2
36	2/5	3	0
37	0/5	0	5
38	0/5	2	3
39	0/5	1	4
40	0/5	5	0
41	0/5	1	4

* Thirty days after inoculation

† Not a donor for next passage

‡ One of these chicks was donor for next passage

examined for parasitemia. On the same day one of the chicks was used as the donor for the next passage. Compression smear preparations were made of the brain cortex

and overlying leptomeninges from each chick that died or was destroyed. These smears were stained with Giemsa and examined for the presence and density of exoerythrocytic parasites. Surviving chicks were killed 30 days after inoculation. Cause of death was tabulated under two categories; (1) parasitemia (with no detectable exoerythrocytic parasites); (2) exoerythrocytic (phanerozoite) infection.

TABLE 3

Series B-1. Blood-passage of the M strain of Plasmodium gallinaceum

BLOOD PASSAGE NUMBER	NO. CHICKS SURVIVED* / NO. CHICKS INFECTED	NO. CHICKS DIED WITH NO PHANEROZOITES	NO. CHICKS DIED WITH PHANEROZOITES
22	2/5	2	1†
23	4/5	1	0
24	2/5	3	0
25	5/5	0	0
26	5/5	0	0
27	4/5	1	0
28	4/5§	1	0
29	5/5§	0	0
30	2/5	3	0
31	5/5	0	0
32	2/5	2	1†
33	2/5	3	0
34	3/5	1	1†
35	2/5	3	0
36	4/5	1	0
37	0/5	5	0
38	5/5	0	0
39	1/5	4	0
40	4/5	1	0
41	3/5	2	0
42	4/5	1	0
43	0/5	3	2‡
44	0/5	3	2
45	1/5	2	2
46	2/5	2	2
47	0/5	5	0
48	0/5	4	1
49	0/5	5	0

* Thirty days after inoculation

† Not a donor for next passage

‡ One of these chicks was donor for next passage

§ Chick with 3+ phanerozoite infection at autopsy, 30 days after inoculation

EXPERIMENTAL

Series A (table 1) was begun on 2 December 1948 after we had made three consecutive mosquito passages of the M strain. In the first 22 passages of this series, 112 (56 per cent) of the infected chicks survived parasitemia; of these, one chick (0.5 per cent of all chicks and 0.8 per cent of all chicks which survived parasitemia) subsequently died of a phanerozoite infection. From the 23rd to the 37th passage,

22 (27 per cent) of the 81 infected chicks survived parasitemia; of these, 19 (23 per cent of all chicks and 86 per cent of all chicks which survived parasitemia) died of phanerozoite infections.

One of the chicks of the 26th passage was used to infect *Aedes aegypti* mosquitoes. Fourteen days later 1.0 mosquito equivalent of sporozoites from these mosquitoes was inoculated into 30 chicks. Each chick developed a typical parasitemia and 28 died with abundant phanerozoites present in the capillary endothelium of the brain. The strain resembled the SP strain as described by Coatney *et al.*, 1945, and Trembley *et al.*, 1951 and 1951a.

TABLE 4

Series C. Blood-passage of the M strain of Plasmodium gallinaceum

BLOOD PASSAGE NUMBER	NO. CHICKS SURVIVED* / NO. CHICKS INFECTED	NO. CHICKS DIED WITH NO PHANEROZOITES	NO. CHICKS DIED WITH PHANEROZOITES
1	5/5	0	0
2	3/5	2	0
3	1/5	4	0
4	1/5	4	0
5	1/5	4	0
6	0/5	5	0
7	3/5	2	0
8	5/5	0	0
9	3/5	2	0
10	4/5	1	0
11	0/5	5	0
12	5/5	0	0
13	2/5	3	0
14	5/5	0	0
15	1/5	1	3†
16	0/5	3	2
17	0/5	4	1
18	0/5	5	0
19	0/5	4	1
20	0/5	4	1

* Thirty days after inoculation

† Chick used as donor for next passage

Series B (table 2) was begun on 20 July 1949 after 12 consecutive mosquito passages. In the first 34 passages of this series, 109 (64 per cent) of the 170 infected chicks survived parasitemia; of these, four (3 per cent of all chicks and 4 per cent of all chicks which survived parasitemia) died of a phanerozoite infection. From the 35th to the 41st passages, 22 (63 per cent) of the 35 infected chicks survived the parasitemia; of these, 18 (51 per cent of all chicks and 82 per cent of all chicks which survived parasitemia) died of phanerozoite infections.

The chick which died of a phanerozoite infection in the 22nd passage of the B series had been used previously as a donor for the 23rd passage of that series. It was anticipated that this might result in an immediate change in the characteristics of the strain. Therefore, one of the surviving chicks of the 22nd passage was used as a

donor for five additional chicks, thus initiating series B-1 (table 3). The passages in this series have been numbered consecutively from passage 22.

From the 22nd to the 42nd passages of this series, 68 (65 per cent) of the infected chicks survived the parasitemia; of these, three (3 per cent of all the chicks and 4 per cent of all chicks which survived parasitemia) subsequently died of a phanerozoite infection. It should be noted that two chicks, killed on the 30th day, apparently in good health, had abundant phanerozoites in the brain capillaries. From the 43rd to the 49th passages, 12 (34 per cent of the infected chicks) survived parasitemia; of these, nine (26 per cent of all the chicks and 75 per cent of all chicks which survived parasitemia) died of a phanerozoite infection.

Series C (table 4) was begun on 20 September 1949, after 15 consecutive mosquito passages. In the first 14 passages of this series, 38 (54 per cent) of the 70 infected chicks survived parasitemia. None of these subsequently died of a phanerozoite infection. From the 15th to the 20th passages, nine (30 per cent) of the infected chicks survived parasitemia; of these, eight (27 per cent of all the chicks and 90 per cent of all the infected chicks which survived parasitemia) died of phanerozoite infections.

DISCUSSION

In each of the four series in which the phanerozoiteless M strain of *P. gallinaceum* was transferred consecutively by the inoculation of infected blood, a marked change occurred in the characteristics of the strain. The early blood passages were characterized by a relatively high rate of survival (61 per cent) and a low rate of deaths (1.6 per cent of all chicks and 2.2 per cent of all chicks which survived parasitemia) from phanerozoite infections.

After a varying number of blood transfers in the four series (14 to 42, mean 29) fewer chicks survived the period of acute parasitemia (mean, 39 per cent) and more chicks which survived parasitemia subsequently died of a phanerozoite infection (mean, 87 per cent). It is notable that whereas the sporozoites of the M strain characteristically produce an infection practically free of phanerozoites the sporozoites of the altered strain produced an infection in which phanerozoites were abundant. Furthermore, the M strain has remained unaltered in its ability to produce phanerozoites through 22 consecutive cyclical mosquito-chick cycles in our laboratory. Prior to our receiving the infection Haas *et al.* (1948) had carried the strain three years by consecutive mosquito-chick passages. Assuming that the latter investigators passaged the infection once each month, the strain would have undergone 58 consecutive cyclical mosquito-chick passages without any change in its ability to produce phanerozoites.

An exoerythrocytic infection occurred in the blood-passaged M strain in 10 chicks of the 362 in which it could have been observed. Chicks which died during the period of acute parasitemia have been excluded because they died before one could tell whether phanerozoites were present. Also excluded are chicks which died of exoerythrocytic infections after such infections had become established in the strain. Thus about four chicks in every hundred independently developed exoerythrocytic infections. It can be conservatively estimated that a chick which survives a *P. gallinaceum* infection for 30 days will have harbored approximately 10^{10} parasites. A

successful invasion and adaptation to survival in the fixed tissues would then have occurred about four times in 10^{12} parasites. This would be the frequency of occurrence of a rare mutant and such mutants probably occur at any time during the course of an infection. For example, in series B, a chick which subsequently died of an exoerythrocytic infection was used as a donor for the 22nd passage; yet there was no evidence of an exoerythrocytic infection either in the 23rd passage or in the 12 subsequent passages. In this case the mutation, if such it is, could have occurred after the chick was used as a donor, or the offspring of the mutant were too few at the time of subinoculation to have been represented in the inoculum transferred to the five recipient chicks.

Concomitant with the development of phanerozoites in the fixed tissue, there was a marked increase in the number of chicks which died during the phase of acute parasitemia. Prior to the development of consistent exoerythrocytic infections, 61 per cent of the chicks survived this early critical period while after the establishment of the exoerythrocytic pattern, only 39 per cent survived long enough for exoerythrocytic infections to become apparent. This might suggest that the host was less resistant to the mutant and consequently the parasitemia was more fulminating, or that the host was weakened by the very early development of tissue parasites in numbers too few to be detected by the means we have employed.

SUMMARY

In each of four independent series a phanerozoiteless M strain of *Plasmodium gallinaceum* was passaged by consecutive weekly blood transfers. In each series an abrupt change in the behavior of the characteristics of the strain occurred after from 15 to 42 blood transfers. The rate of survival, initially high, was markedly reduced, and 87 per cent of the chicks which survived parasitemia subsequently died of phanerozoite infections. The ability to invade and reproduce in the fixed tissue was maintained through mosquito passage.

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RESUMEN

En cada una de cuatro series independientes una cepa M, no fanerozoíta, de *Plasmodium gallinaceum* fué transferida semanalmente a través de pases de sangre consecutivos. En cada serie ocurrió un cambio repentino en el funcionamiento de las características de la cepa después de 15 a 42 pases de sangre. La proporción de sobrevivientes, inicialmente alta, se redujo considerablemente y 87 porciento de los pollos que sobrevivieron la parasitemia luego murieron de infecciones fanerozoítas. La abilidad para invadir y reproducirse en el tejido fijo se mantuvo por medio de pases en mosquitos.

33RD ANNUAL MEETING

OF THE

NATIONAL MALARIA SOCIETY

Held Conjointly with the American Society of Tropical Medicine and the American Academy of Tropical Medicine

MINUTES—1950

Officers

President—Dr. Paul F. Russell, New York, New York
President-Elect—Dr. Justin M. Andrews, Atlanta, Ga.
Vice-President—Mr. W. H. W. Komp, College Park, Md.
Secretary-Treasurer—Dr. Martin D. Young, Columbia, S. C.

Directors:

Dr. Lloyd E. Rozeboom, Baltimore, Maryland
Dr. E. L. Bishop, Chattanooga, Tennessee
Mr. H. W. Van Hovenberg, Mt. Pleasant, Texas
Dr. Wendell Gingrich, Galveston, Texas—Past President

Tuesday, November 7, 1950

The National Malaria Society convened for its 33rd annual meeting at 8:40 A.M., in the Grand Ballroom of the Hotel DeSoto in Savannah, Georgia, with President Paul F. Russell presiding. At the conclusion of the President's address, entitled "Malaria and Society", 11 papers were read and two presented by title. At the end of the scientific program a business session was held, which is reported below.

Wednesday, November 8, 1950

The scientific session re-convened at 2:00 P.M. to hear the symposium entitled "Nation-wide Malaria Eradication Projects in the Americas". Dr. Paul F. Russell, acting as moderator, introduced the symposium and the individual speakers: Dr. Justin M. Andrews, Dr. Arnoldo Gabaldon, Dr. George Giglioli and Dr. Fred L. Soper. Dr. Mario Pinotti of Brazil was absent but a summary of his paper was presented by Dr. Henry Kumm. The session adjourned at 5:15 P.M.

Thursday, November 9, 1950

The Society re-convened at 2:00 P.M. for a conjoint meeting with the American Society of Tropical Medicine, during which the Presidents-Elect of the two societies presided. A program of 12 papers was presented. The meeting adjourned at 5:20 P.M.

General

On Monday, November 6, 1950, registration was begun. The Technical Development Services, Communicable Diseases Center, U. S. Public Health Service, held an Open House and conducted tours of its laboratories on Oatland Island.

Through the efforts of the local committee on arrangements, the following entertainment was sponsored: hospitality sessions on Monday, Tuesday and Wednesday from 5 until 7 P.M.; entertainment for visiting ladies including tours, luncheon and a speaker; a dance on Tuesday evening; a deer hunt and fishing trips on Friday.

Minutes of the Business Meeting

November 7, 1950

The minutes of the 1949 annual meeting in Memphis, Tennessee, were approved as published in the Journal of the National Malaria Society in March 1950. The Secretary-Treasurer reported as follows:

From the 1949 roster of 567 active members, three (Dr. Banner Bill Morgan, Dr. C. F. Adams, and Mr. Thomas A. Randle) have been lost by death, 12 have resigned, and 44 have been dropped because of delinquency in dues. During the year 19 new members have been elected, making an active membership of 527. This represents a loss of 40 members. Of the membership, 416 were in good standing on October 31, 1950.

The status of the treasury at the close of business October 31, 1950, was:

Balance reported October 31, 1949	$5,582.74
Receipts from delinquent, current, and advance dues, subscriptions, sales of back issues, advertising, interest and miscellaneous	4,976.38
Total	$10,559.12
Expenditures before paying for the fourth issue of the 1950 Journal, but including the cost of the fourth issue of the 1949 Journal	5,031.45
Balance on hand October 31, 1950	$5,527.67

Of the balance on hand October 31, 1950, $5,036.39 is in the Publication Fund and $491.28 is in the Operating Fund.

Col. Tom F. Whayne reported for the Committee on Auditing, stating that the books and accounts of the Secretary-Treasurer had been examined and found in order and commending the practice of having the report accompanied by an audit made by a Certified Public Accountant. The Committee recommended that honoraria in the amount of $275 to the Secretary's stenographer and $35 to the Editor's stenographer be granted. The Society adopted the report and recommendations of the Auditing Committee, at the same time adopting the report of the Secretary-Treasurer.

The report of the meeting of the Board of Directors on November 7, 1950, was read and accepted. The minutes of this meeting are printed elsewhere in the Journal.

In the absence of the Chairman of the Committee on *Plasmodium berghei* (Dr. L.

T. Coggeshall), the Secretary gave a brief report which indicated that this parasite could be released to laboratories which meet the strict quarantine regulations of the Department of Agriculture. This report was accepted.

Dr. George H. Bradley, in reporting for the Committee on Lectureship and Award, said that no definite plans had materialized during the present year for the presentation of the award but that it was hoped to secure more definite plans during the coming year. This report was accepted.

Dr. G. Robert Coatney, reporting as Representative of the AAAS Council, indicated that one of the main activities of the Council was the discussion over mailing the programs to the members so they would receive them in advance of the meetings.

For the Committee on Resolutions, Dr. Walter C. Earle introduced resolutions, expressing the Society's profound regret upon the death of Dr. Banner Bill Morgan, Dr. C. F. Adams, and Mr. Thomas A. Randle, and instructed the Secretary to communicate an expression of sympathy to the families concerned. Other resolutions conveyed the appreciation of the Society to the Committee on Local Arrangements; to the several host organizations consisting of the Technical Development Services, the Communicable Disease Center, the Savannah-Chatham County Health Department, and the Medical Association of Georgia; to the commercial companies sponsoring the hospitality sessions, the dance, the ladies' entertainment program, the fishing trip and the deer hunt. The Federal Government was entreated to provide funds to enable the Fifth World Health Assembly to hold its 1952 meeting in the United States.

Dr. Wendell Gingrich introduced a resolution calling for a rising vote of thanks to the outgoing Secretary for his 5 years of service to the Society.

All the above proposed resolutions were adopted by the Society.

The Tally Committee reported that, as a result of the votes counted, the following officers were elected for 1951:

President-Elect—Mr. W. H. W. Komp
Vice President—Dr. E. L. Bishop
Director for 3 years—Dr. George H. Bradley

Dr. A. J. Walker suggested that a complete membership list be printed in an early issue of the Journal.

Dr. Fred L. Soper stated that the XIIIth Pan American Sanitary Conference approved the commemoration of the 50th Anniversary of the founding of the First International Health Organization by the convocation, in collaboration with the Government of Cuba, of the First Inter-American Sanitary Congress in the City of Havana, Cuba, in 1952.

A definite date for this Congress has not been set but will undoubtedly be late in November or early in December. Dr. Soper suggested that the National Malaria Society and the American Society of Tropical Medicine should consider holding their Annual Meetings in Havana at the time of this Sanitary Congress. Official invitations to these Organizations will be forthcoming from the Pan American Sanitary Bureau, in due course.

This ended the business meeting which was re-convened for a very short session immediately after the Symposium on November 8, at 5:15 P.M. At this time the report by the Committee on Criteria of Malaria Endemicity was presented by Dr. E. Harold Hinman. A mimeographed copy of this report had been circularized to the membership previously. The Society accepted the recommendations of the Criteria Committee and it was suggested that the report be published in the Journal of the National Malaria Society in the near future.

Mr. Frederick L. Knowles, Editor of the Journal for 5 years, received the thanks and commendations of the Society for his faithful and untiring efforts during his term of office and for the excellence of the publication. Dr. G. Robert Coatney was named as the incoming Editor of the Journal of the National Malaria Society.

The business meeting adjourned sine die at 5:20 P.M.

MEETING OF THE BOARD OF DIRECTORS, NATIONAL MALARIA SOCIETY

Monday, November 6, 1950, Hotel DeSoto, Savannah, Georgia

The Board of Directors of the National Malaria Society met at 3:00 P.M. November 6, 1950, in the Habersham Room of the Hotel DeSoto, Savannah, Georgia. Members present were Dr. Paul Russell, President; Dr. Justin M. Andrews, President-Elect; Mr. H. W. Van Hovenberg, Director; Dr. Martin D. Young, Secretary; and Mr. Frederick L. Knowles, Editor. Dr. Quentin M. Geiman, Secretary-Treasurer of the American Society of Tropical Medicine, was present by invitation.

Dr. Russell appointed committees to canvass the ballots for election of officers, to audit the books of the Secretary, and to draw up appropriate resolutions.

The actions of the Board of Directors during 1950 were reviewed as follows: approved committee appointments made by President Russell; did not approve Dr. Sowder's proposal that the National Malaria Society enter the controversy about impoundment regulations because this is a matter of the application of State laws and Army Engineers' policy; some Directors wrote to State electors of the New York University Hall of Fame supporting the nomination of General Gorgas; did not approve entering a Federation of Microbiologists, which includes bacteriologists and immunologists, for the purpose of conducting joint meetings; elected 19 members for 1950 and 3 for 1951; approved expenses of the Editor to the meeting up to $50.

The report of the Secretary-Treasurer was accepted.

The report of the Editorial Board was accepted. The Directors authorized the printing of 96 pages per quarterly issue in the 1951 Journal.

The report of the Committee on Lectureship and Award, by Dr. George H. Bradley, stated that at present there were no funds to underwrite a cash award which had been proposed to be given with the LePrince Medal. The Board of Directors expressed itself as being in favor of proceeding in the near future with presenting the medal, even if no monetary award could accompany it.

The Directors approved the presenting of the report of the Committee on Criteria to Determine When Malaria Ceases to be an Endemic Disease to the business meeting of the National Malaria Society.

It was felt that the Committee on Statistics needed to be continued. Dr. Faust's request that he no longer serve as chairman of this committee was granted. The Board went on record with generous thanks to Dr. Faust for his long and valuable service to the Society during the 15 years he so faithfully prepared the reports on malaria statistics.

It was moved that the Society join other groups in supporting the action to have the Fifth World Assembly meet in the United States in 1952.

The resignation of Dr. Martin D. Young as Secretary-Treasurer was accepted with regret and with abundant thanks for the way he has handled the duties of the office for the past 5 years. Dr. Samuel W. Simmons was elected as the new Secretary-Treasurer. The Board authorized the Secretary to continue a $5,000 bond during

1951. Approval was granted for the Secretary to handle all routine business for the Society.

The Directors approved honoraria in the amount of $275 for the Secretary's stenographer and $35 for the Editor's stenographer for their services during the year 1950.

Responsibility was deputized to the Secretary to meet with the Secretaries of the other Societies for the purpose of selecting a time and place for the 1951 meeting.

In discussing the question of the future of the Society it was pointed out by Dr. Russell that the Society could make one of three choices as to its destiny, each choice having something to recommend it: 1) the Society could dissolve at some future time when malaria has been declared as ceasing to be an endemic disease in this country. This would be a unique procedure in the annals of scientific societies, as our society would become the first society to achieve its designated goals; 2) the interests of the Society could be broadened and the name changed to one indicating that it is an organization interested in arthropod-borne diseases. By this procedure, the study of the diseases borne by arthropods would be incorporated in the Society, thus continuing the very successful pattern set up by the National Malaria Society whereby biologists, physicians and engineers have worked together as a competent and cooperative team in the attack upon a single disease. Such team work, engendered by the understanding and acceptance of the importance of each field of knowledge, is responsible for the great advances made in the field of malaria, and undoubtedly could be used to tremendous advantage in the larger field of arthropod-borne diseases. It is believed that the spirit of cooperation exhibited by the various professions working together has resulted in the very cohesive spirit so characteristic of the National Malaria Society. 3) The National Malaria Society could join with the American Society of Tropical Medicine thus uniting the two organizations into one society of broad interests. The National Malaria Society has in common with the American Society of Tropical Medicine a membership of about 200 persons. As the interests of these two societies tend to overlap, a merger seems to have many advantages, if precautions are taken to respect the interests of both groups.

The Board voted in favor of the principle of the amalgamation of the National Malaria Society with the American Society of Tropical Medicine, and approved a committee of four to meet with a similar committee of the American Society of Tropical Medicine to investigate the possibility of amalgamation and to work out general plans. Appointed to this committee were the President, Dr. Justin M. Andrews; the outgoing Secretary, Dr. Martin D. Young; the incoming Secretary, Dr. Samuel W. Simmons; and a fourth member, Mr. John M. Henderson.

The meeting adjourned sine die at 5:25 P.M.

SCHEDULE OF LABORATORY TRAINING COURSES
Jan. 1 to Dec. 31, 1951

Feb. 12–23—Laboratory Diagnosis of Syphilis
Feb. 26 to Mar. 2—Microbiology for Public Health Nurses
Feb. 26 to Mar. 9—Laboratory Diagnosis of Bacterial Diseases. General Bacteriology, Part 1
Mar. 5–23—Laboratory Diagnosis of Parasitic Diseases. Part 1. Intestinal Parasites
Mar. 12–23—Laboratory Diagnosis of Bacterial Diseases. General Bacteriology, Part 2
Mar. 12–23—Laboratory Diagnosis of Syphilis
Mar. 26 to Apr. 13—Laboratory Diagnosis of Parasitic Diseases. Part 2. Blood Parasites
Mar. 26–30—Laboratory Diagnosis of Enteric Diseases. Part 1. Introductory Enteric Bacteriology
Apr. 2–13—Laboratory Diagnosis of Enteric Diseases. Part 2. Advanced Enteric Bacteriology
Apr. 16–27—Laboratory Diagnosis of Mycotic Diseases. Part 1. Cutaneous and Subcutaneous Fungi
Apr. 16–27—Laboratory Diagnosis of Tuberculosis
Apr. 16–27—Laboratory Diagnosis of Syphilis
Apr. 16 to May 11—Laboratory Diagnosis of Virus Diseases
Apr. 30 to May 11—Laboratory Diagnosis of Mycotic Diseases. Part 2. Systemic Fungi
Apr. 30 to May 11—Laboratory Diagnosis of Tuberculosis
May 7–11—Laboratory Diagnosis of Venereal Diseases*
May 14–18—Laboratory Diagnosis of Mycotic Diseases*
May 14–18—Laboratory Diagnosis of Tuberculosis*
May 14–18—*Treponema pallidum* Immobilization*
May 14–18—Laboratory Diagnosis of Rabies
May 14–18—Clinical Chemistry. Part 1. Introductory and General Procedures
May 21–25—Laboratory Diagnosis of Parasitic Diseases*
May 21–25—Laboratory Diagnosis of Bacterial Diseases*
May 21–25—Laboratory Diagnosis of Virus Diseases*
May 21 to June 1—Clinical Chemistry. Part 2. Quantitative Analyses
June 4–15—Laboratory Diagnosis of Syphilis
Aug. 27–31—Microbiology for Public Health Nurses
Aug. 27 to Sept. 7—Laboratory Diagnosis of Bacterial Diseases. General Bacteriology, Part 1
Sept. 3–21—Laboratory Diagnosis of Parasitic Diseases. Part 1. Intestinal Parasites

* These courses are designed for directors.

Sept. 3–28—Laboratory Diagnosis of Virus Diseases
Sept. 10–21—Laboratory Diagnosis of Bacterial Diseases. General Bacteriology, Part 2
Sept. 10–21—Laboratory Diagnosis of Syphilis
Sept. 24 to Oct. 12—Laboratory Diagnosis of Parasitic Diseases. Part 2. Blood Parasites
Sept. 24–28—Laboratory Diagnosis of Enteric Diseases. Part 1. Introductory Enteric Bacteriology
Oct. 1–12—Laboratory Diagnosis of Enteric Diseases. Part 2. Advanced Enteric Bacteriology
Oct. 1–5—Laboratory Diagnosis of Rabies
Oct. 8–12—Laboratory Diagnosis of Virus Diseases*
Oct. 22–26—Laboratory Diagnosis of Parasitic Diseases*
Oct. 22–26—Laboratory Diagnosis of Bacterial Diseases*
Oct. 22 to Nov. 2—Laboratory Diagnosis of Syphilis
Oct. 29 to Nov. 2—Laboratory Diagnosis of Mycotic Diseases*
Oct. 29 to Nov. 2—Laboratory Diagnosis of Tuberculosis*
Oct. 29 to Nov. 2—Clinical Chemistry. Part 1. Introductory and General Procedures
Nov. 5–16—Laboratory Diagnosis of Mycotic Diseases. Part 1. Cutaneous and Subcutaneous Fungi
Nov. 5–16—Laboratory Diagnosis of Tuberculosis
Nov. 5–23—Preparation and Standardization of Serologic Reagents Used in the Laboratory Diagnosis of Syphilis
Nov. 5–16—Clinical Chemistry. Part 2. Quantitative Analyses
Nov. 19–30—Laboratory Diagnosis of Mycotic Diseases. Part 2. Systemic Fungi
Nov. 19–30—Laboratory Diagnosis of Tuberculosis

By Special Arrangement

Laboratory Diagnosis of Malaria
Identification of Medically Important Arthropods
Typing of *Corynebacterium diphtheriae*
Phage Typing of *Salmonella typhosa*
Serologic Diagnosis of Rickettsial Diseases
Virus Isolation and Identification Techniques
Laboratory Diagnosis of Influenza
Advanced Quantitative Analyses in Clinical Chemistry
Toxicology

Information and application forms should be requested from the Officer in Charge, Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.